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Isolation and Characterization of Efficient Phosphate Solubilizing Bacteria from the Rhizosphere Soil of *Tephrosia Purpurea* Linn.

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A B S T R A C T

Agricultural crops require phosphorus as a major macronutrient for its better growth and yield. Though the phosphorus that is available in soil and the added phosphorus in the form of fertilizers will not be able to fully utilize by the crops due to the insoluble form of phosphorus. So, the major thrust of the research work is to convert the insoluble form of phosphate in to soluble as well as easily accessible form to the crops, for that indigenous phosphate solubilzing bacteria are collected from the rhizosphere soil of *Tephrosia purpurea* which is one of the green manure crop and studied their efficiency for the betterment of growth and development of agriculture crops. Totally 86 strains of phosphate solubilizing bacteria have been collected from the different locations in Tamil Nadu by plating in Pikovskaya medium. Among them 10 efficient strains were selected which shows the greater zone of inhibition and for these strains morphological; biochemical studies and pH reduction were performed. Out of these 2 strains (PVJ 1 and PVJ 5) were selected based on the maximum zone of inhibition (total diameter) of 49 mm and 51 mm and shows the fastest reduction in pH from 7 to 2.5 and 2.3 respectively within 3-4 days were further subjected to FITR and GCMS analysis. From the analysis the compounds such as Benzenepropanoic acid, Benzenedicarboxylic acid, Phosphonic acids and Sebacic acid has the antimicrobial properties that produced by both the bacteria involved in the mechanism of phosphate solubilization by chelating the iron present in the medium in the form of Calcium phosphate and ferrous phosphate thereby reducing the pH of the medium and formation of zone. On application of these strains as a biofertilizer it results in good growth and yiel9d due to their efficiency in phosphate solubilization and their compounds exhibits antimicrobial activity against the plant pathogens.

***Key words*:** Phosphate solubilizing, *Tephrosia purpurea,* Zone of inhibition, Efficient bacterial strain, FTIR and GCMS

Agriculture plays a significant role in everyone's life. Agricultural crops successfully utilize the nitrogen in higher level followed by the moderate level of potassium, at the same time it struggles to utilize the phosphorus. In order to rectify these issues farmers incorporate phosphorus fertilizers in the fields. Although, phosphorus is an important macronutrient, it is essential for better plant maturation, photosynthesis, root establishment, energy transfer, good flowering [1] but 95-99% of it, present in the soil is insoluble and cannot be utilized by plants [2]. This is due to either it is adsorbed on the soil minerals or get precipitated by free Al^{3+} and Fe^{3+} in the soil solution [3-4] which is highly reactive in acidic and Ca^{2+} in calcareous soils [5].

The term Phosphorus solubilization indicates the solubilization of inorganic phosphorus whereas Phosphorus mineralization refers to the solubilization of organic phosphorus. Basically, inorganic phosphate was divided into five major groups such as soluble phosphate, aluminum phosphate, iron phosphate, calcium phosphate and occluded phosphate. Except the soluble phosphate the other phosphates are exist with hard to dissolve forms [6]. Only negatively charged primary and secondary orthophosphate ions ($H_2PO_4^-$ and HPO_4^{2-}) are utilized by the plants. The insoluble forms of phosphorus such as tricalcium phosphate, aluminum phosphate or iron phosphate may be converted to soluble phosphate by phosphate-solubilizing microorganisms and enzymes (e.g., phosphatase,

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phosphotriesterases) that are present in soils [7]. Phosphorus solubilizing microbes exhibit multifunctional activities that benefits plants by synthesizing siderophores, indole acetic acid and gibberellic acid etc. [8]. Another mechanism of phosphate solubilization by microbes is by the production of inorganic acids and the production of chelating substances. It has been reported that due to the effectiveness of the inorganic acids and the chelating substances in the release of than that of the organic acids.

Tephrosia purpurea (Linn) Pers, (Leguminasae) is a polymorphic, much branched sub erect perennial herb popularly known as "Sarapunkha" in Sanskrit, "Purple Tephrosia" in English and "Kaattukolingi" in Tamil. *Tephrosia purpurea* (Linn.) Pers, belongs to the family Fabaceae, subfamily Faboideae, tribe Millettieae, and it is a highly branched suberect herbaceous perennial, up to 60 m in height with spreading branches; the leaves are narrow imparipinnate, the flowers are Lavender or purple colour in extra-axillary racemes, the pods are slightly curved, 3 – 4.5 cm long, grey, smooth and containing 5–10 seeds per pod. It is a highly branched, sub – erect perennial herb [9]. In many parts it is under cultivation as green manure crop. It is found throughout India and Sri Lanka in poor soils. The herb is commonly grown as a green manure in paddy fields in India and in tobacco and rubber plantation in other countries. It grows ubiquitously in all soils, sandy, rocky and loamy [10].

Taxonomy

Kingdom : Plantae
Order : Fabales
Family : Fabaceae
Genus : *Tephrosia*
Species : *purpurea*

The objectives of this research were to isolate and identify the efficient Phosphate solubilizing bacteria from the rhizosphere soil of *Tephrosia purpurea* and to study their efficiency as a biofertilizer for the betterment of growth and development of agriculture crops.

MATERIALS AND METHODS

Sample collection

Thirty rhizosphere soil samples of *Tephrosia purpurea* L. plants were collected from different locations in Tamil Nadu. The Rhizoshere soil samples which adhere to the root hairs and about 0-15 cm below the surface soil of *Tephrosia purpurea* (Linn.) plants. The samples were collected randomly from the agricultural fields and bushy areas within 1-2cm interval between the same samples and air dried, sieved and stored in sterile plastic containers.

Plant collection

Plant material of *Tephrosia purpurea* (Linn.) were collected from different parts of Tamil Nadu. The plant material (from Trichy Region) was authenticated at the Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph College, Tiruchirapalli-2, Tamil Nadu, India. A voucher specimen as an herbarium authenticated as K.V.001 has been kept for future reference.

Isolation of phosphate solubilizing bacteria

The soil sample were serially diluted in sterile distilled water from 10^{-1} to 10^{-7}. The initial dilution was prepared by adding sample into 9 ml dilution blank labeled 10^{-1}. From the first dilution 1ml of the suspension was transferred to dilution blank 10^{-2}. This procedure was repeated till the original sample has been diluted 10^{-7} times, using every time a fresh sterile pipette. Diluted soil sample 10^{-4}, 10^{-5} and 10^{-6} were inoculated in Pikovskaya agar medium [11] and incubated at 37°C for 2-7 days. Triplicates were done and the uninoculated plate served as control.

Characterization of isolated bacterial strains

From the collected soil sample a total number of 19 phosphate solubilizing bacterial isolates were selected on the basis of zone formation and designated as PVJ 1-PVJ 10. Morphological characteristics of each isolated colony were examined on pikovskaya plates. After 7 days of incubation, different characteristics of colonies such as size, shape, elevation, margin, opacity, pigmentation, etc. were recorded [12].

Screening for phosphate solubilization

Phosphorus solubilization of phosphate solubilizing bacterial strains was screened on Pikovskaya agar plates for determining their solubilizing activity by measuring the phosphate solubilization index (SI). The halo zone formation of the bacteria was quantitatively determined by the method of Edi-Premono (1996). All the observations were recorded in triplicate.

$$SI = \frac{\text{Colony diameter + Halozone diameter}}{\text{Colony diameter}}$$

Effect of pH on efficiency of phosphate solubilization

Phosphate solubilizing efficiency was analyzed by the change in the pH of broth media. For this the identified efficient phosphate solubilizing bacterial culture was inoculated in Pikovskaya broth and incubated for 14 days. The initial pH and the change in pH were checked and recorded from day 1 to day 14 of incubation [13].

Biochemical characterization of selected bacterial strains

The isolated efficient strains PVJ 1 and PVJ 5 were identified using standard biochemical tests as in the Bergey's Manual of Determinative Bacteriology [12].

FITR Spectrum Analysis

FTIR relies on the fact that the most molecules absorb light in the infrared region of the electromagnetic spectrum. This absorption corresponds specifically to the bonds present in the molecule. The frequency ranges are measured as wave numbers typically over the range 4000-600cm^{-1}. The compounds were analyzed using Shimaze IR affinity I instrument.

Gas Chromatography –Mass Spectroscopic Analysis

Gas chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one being a stationary bed of large surface area and the other, a gas that percolates through the stationary bed. GC is used to separate a wide variety of volatile organic compounds. GC-MS analysis of the bacterial sample was performed using the equipment Thermo GC-Trace Ultra version: 5.0, Thermo MS DSQ II. The equipment has a DB 35-MS capillary standard non-polar column with dimension of 30 mm × 0.25 mm ID × 0.25 mm film. The carrier gas used is helium with at low of 1.0 ml/min. The injector was operated at 250°C and the oven temperature was programmed as 60°C for 15 mins and

gradually increased to 280°C at 3 min (13). The compounds are identified based on NIST libraries as well as comparison of their retention indices.

RESULTS AND DISCUSSION

A total number of 86 isolates were isolated from the rhizosphere soil samples of *Tephrosia purpurea* (Linn.) plants from the different parts of Tamil Nadu. Based on their ability of producing the zone formation, the colonies were selected and maintained in pure culture in Pikovskaya medium. 10 isolates were chosen based on the formation of zone and numbered as PVJ1 to PVJ 10. Morphological and phosphate solubilization efficiency were carried out for the isolates and presented in (Table 1-2).

Table 1 Colony morphology and microscopic observation of isolated bacterial strains

Isolate	Size	Shape	Margin	Elevation	Surface texture	Consistency	Optical Character	Pigmentation
PVJ 1.	Moderate	Irregular	Lobate	Convex	Rough	Moist	Translucent	Yellow
PVJ 2.	Moderate	circular	Serrate	Umbonate	Rough	Moist	Opaque	Yellowish white
PVJ 3.	Big	circular	Entire	Convex	Glistening	Butyrous	Translucent	Brownish
PVJ 4.	Large	Irregular	Undulate	Umbonate	Smooth	Moist	Translucent	White
PVJ 5.	Moderate	Irregular	Entire	Umbonate	Smooth	Moist	Opaque	Pale White
PVJ 6.	Moderate	Circular	Entire	Umbonate	Rough	Moist	Opaque	White
PVJ 7.	Large	Irregular	Entire	Flat	Glistening	Moist	Translucent	Yellow
PVJ 8.	Moderate	Rhizoid	Lobate	Convex	Contoured	Butyrous	Opaque	Dull white
PVJ 9.	Small	Circular	Entire	Raised	Glistening	Mucoid	Opaque	White
PVJ 10.	Large	Irregular	Undulate	Umbonate	Wrinkled	Moist	Translucent	Dull white

Table 2 Efficiency of Phosphate Solubilization of Isolated Bacterial Strains

Isolate	Colony diameter (mm)	Halozone diameter (mm)	Solubilization index (mm)
PVJ 1	1.6	51.2	33.6
PVJ 2	1.8	49.1	29.1
PVJ 3	2.1	52.6	27.4
PVJ 4	1.2	46.4	39.8
PVJ 5	1.4	56.3	41.6
PVJ 6	1.6	42.3	27.1
PVJ 7	1.7	48.7	30.3
PVJ 8	2.2	46.4	23.2
PVJ 9	2.4	49.8	23.1
PVJ 10	2.1	49.1	25.2

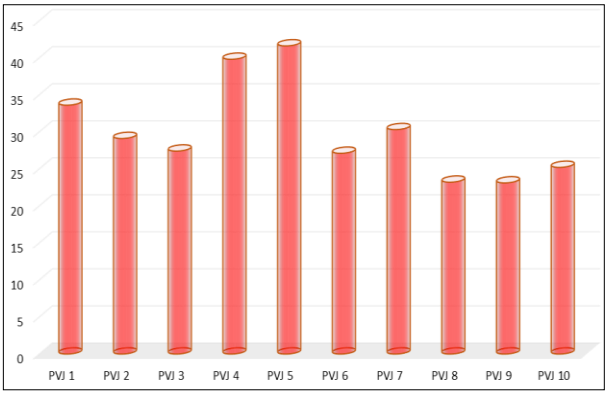


Fig 1 Efficiency of phosphate solubilization of isolated bacterial strains

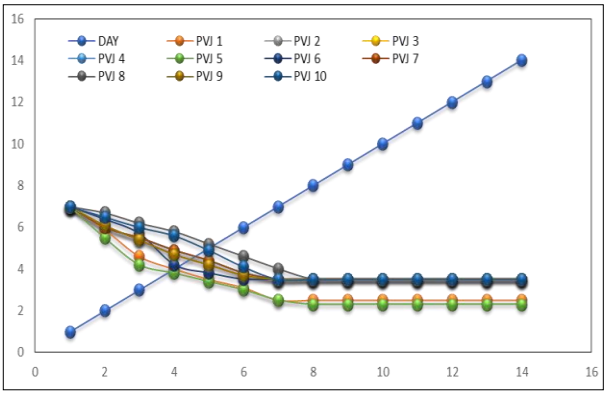


Fig 2 Effect of pH on efficiency of phosphate solubilization

Out of the 10 isolates 5 strains shows the maximum inhibition. The first strain (PVJ1) which has the colony characteristics of yellow colour, medium, irregular shaped with lobate elevation, convex and smooth colony with moist and translucent optical character with the solubilzation index of 33.6mm. Following this the second colony (PVJ5) which are irregular shaped with entire elevation and has an umbonate, smooth moist and translucent optical character with pale white pigmentation exhibits zone within the diameter of 41.6 mm. The Zone of inhibition was calculated for the period of 7 days of incubation after which there was a reduction in Zone diameter. Both the colonies had the faster growth and the Zone of inhibition was noted after 24 hours of incubation itself resulting in decrease of pH of 7 to 5.9 and 5.5. These bacterial isolates were further characterized and screened by biochemical tests and identified as *Bacillus* sp. (Table 3). Screening for the phosphate solubilizing activity of the selected bacterial isolates was done by both qualitatively and quantitatively. The data of (Table 4) shows the quantitative estimation of isolates on PKV broth with initial pH 7.0 respectively. The production of organic acid or chelating the iron by the isolates solubilizes tricalcium phosphate which results in the reduction in pH level. The maximum reduction in pH was noted in 7th day of incubation which falls down to 2.5 and 2.3 respectively. For the qualitative analysis the bacterial

isolates were plated in NBRIP medium supplemented 0.1% bromothymol blue which upon incubation produces maximum zone formation along with the change in colour from blue to yellow colour around the colonies due to the reduction in pH by the isolates. The efficient strains PVJ1 and PVJ5 are further studied by FITR and GCMS analysis. In FITR analysis (Table 5, Fig 4) of PVJI shows 9 peak values and (Table 6, Fig 5) PVJ5 shows 4 peak values. The analysis of compounds of samples PVJ1 shown in (Table 7, Fig 6) Chromatogram GC-MS analysis of PVJ1 showed the presence of twenty-four major components corresponding to the peaks were determined. The analysis of compounds of samples PVJ5 shown in (Table 8, Fig 7) eighteen components corresponding to the peaks were also determined for PVJ5.

All the compounds exhibit to have antimicrobial activities and the compounds from 7 to 2.5 and 2.3 respectively within 3-4 days were further subjected to FITR and GCMS analysis. From the analysis the compounds such as Benzenepropanoic acid, Benzenedicarboxylic acid, Phosphonic acids and Sebacic acid has the antimicrobial properties that produced by both the bacteria involved in the mechanism of phosphate solubilization by chelating the iron present in the medium in the form of Calcium phosphate and ferrous phosphate thereby reducing the pH of the medium and formation of zone.

Table 3 Effect of pH on efficiency of phosphate solubilization

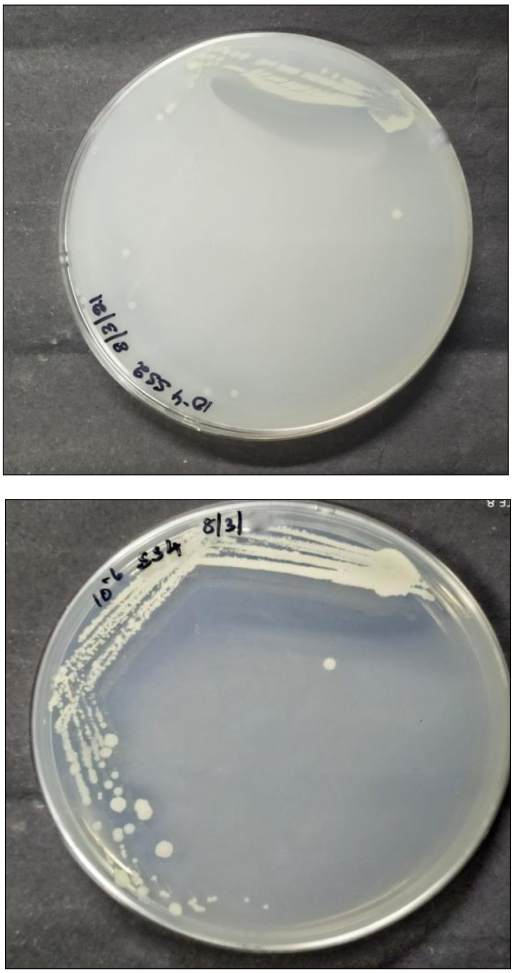
Day	PVJ 1	PVJ 2	PVJ 3	PVJ 4	PVJ 5	PVJ 6	PVJ 7	PVJ 8	PVJ 9	PVJ 10
1	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
2	5.9	6.0	6.1	6.0	5.5	6.4	6.0	6.7	6.1	6.5
3	4.6	5.5	5.4	5.5	4.2	5.7	5.5	6.2	5.4	6.0
4	4.0	4.9	4.7	4.9	3.8	4.2	4.9	5.8	4.7	5.6
5	3.5	4.4	4.2	4.4	3.4	3.8	4.4	5.2	4.2	4.9
6	3.1	3.8	3.7	3.8	3.0	3.5	3.8	4.6	3.7	4.1
7	2.5	3.5	3.5	3.5	2.5	3.5	3.5	4.0	3.5	3.5
8	2.5	3.5	3.5	3.5	2.3	3.5	3.5	3.5	3.5	3.5
9	2.5	3.5	3.5	3.5	2.3	3.5	3.5	3.5	3.5	3.5
10	2.5	3.5	3.5	3.5	2.3	3.5	3.5	3.5	3.5	3.5
11	2.5	3.5	3.5	3.5	2.3	3.5	3.5	3.5	3.5	3.5
12	2.5	3.5	3.5	3.5	2.3	3.5	3.5	3.5	3.5	3.5
13	2.5	3.5	3.5	3.5	2.3	3.5	3.5	3.5	3.5	3.5
14	2.5	3.5	3.5	3.5	2.3	3.5	3.5	3.5	3.5	3.5

Table 4 Biochemical characterization of selected efficient bacterial strains

Tests	PVJ1 (*Bacillus*)	PVJ5 (*Bacillus*)
Gram staining	Gram positive	Gram positive
Motility	Positive	Positive
Indole production	Negative	Negative
Methyl red	Positive	Positive
Voges Proskauer	Negative	Negative
Catalyze	Positive	Positive
Oxidase	Positive	Positive
Urease	Positive	Positive
Citrate utilization	Positive	Positive
Starch hydrolysis	Positive	Positive
Casein hydrolysis	Positive	Positive
Gelatin	Positive	Positive
H_2S production	Negative	Negative
Nitrate reduction	Positive	Positive

Table 5 *FITR spectrum analysis*: *PVJ1*

Peak (Wave number)	Intensity	Bond	Functional group
3447.97 cm^{-1}	Medium, Broad	N-H	Alcohol
2925.10 cm^{-1}	Medium	C-H	Alkane
2853.35 cm^{-1}	Medium	C-H	Alkane
2093.13 cm^{-1}	Medium	C =C	Alkyne
1636.79 cm^{-1}	Medium	C =H	Aromatic compound
1456.16 cm^{-1}	Medium	C-H	Alkane
1415.61 cm^{-1}	Strong, Broad	O -H	Caboxylic acid
1114.44 cm^{-1}	Strong	C -O	Aliphatic ether
649.92 cm^{-1}	Medium	C -I	Halo compound



PVJ1

PVJ5

Fig 3 Efficient bacterial isolates

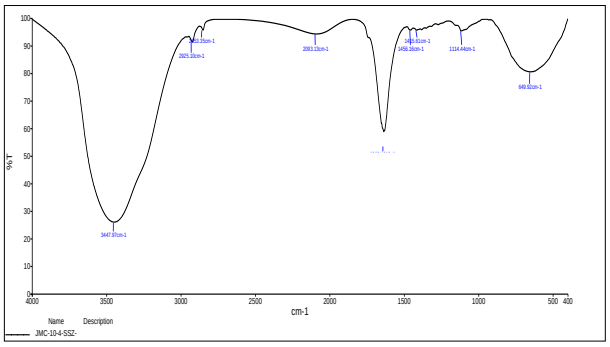


Fig 4 FITR Spectrum analysis of PVJ1

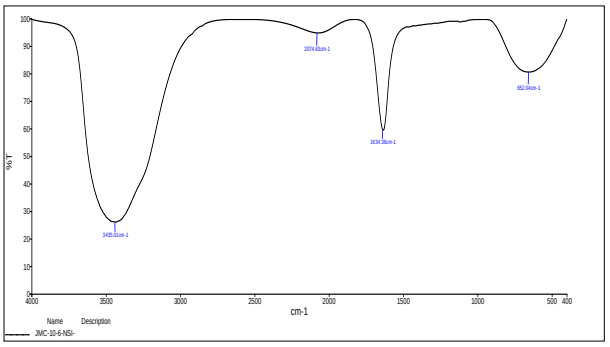


Fig 5 FITR Spectrum analysis of PVJ5

Table 6 FITR Spectrum Analysis: PVJ5

S. No	Peak (Wave Number)	Intensity	Bond	Functional group
1.	3435.01 cm^{-1}	Medium, Broad	N-H	Alcohol
2.	2074.63 cm^{-1}	Strong	C =C=C	Allene
3.	1634.38 cm^{-1}	Medium	C =H	Aromatic compound
4.	652.04 cm^{-1}	Medium	C -I	Halo compound

Table 7 GCMS Analysis of PVJ1

S. No.	Phytochemicals	Molecular weight	Pharmacological actions
1	Dodecane	170	Antimicrobial activity
2	Tetradecane	198	Antimicrobial activity
3	2,4-Di-Tert-Butylphenol	206	Fungicidal activity
4	Hexadecane	226	Antimicrobial activity.
5	Cyclopentane	70	Antimicrobial activity, Anticancer activities
6	P-Octylacetophenone	232	Anti-inflammatory
7	Octadecane	254	Antibacterial activity
8	7,9-Di-Tert-Butyl-1-Oxaspiro	276	Antimicrobial activity, Antioxidant
9	2,5-Cyclohexadien-1	94	Antibacterial activity
10	Hexadecanoic Acid	254	Antioxidant, Hypocholesterolemic, Nematicide,Pesticide,Antiandrogenic, Heamolytic, Anti-inflammatory
11	Benzenepropanoic Acid	179	Antimicrobial activity
12	1,2-Benzenedicarboxylic Acid	276	Antifungal, Antidiarrhoeal and Antifouling activities
13	1-Acetyl-4 -Piperazine	141	Antimicrobial activity
14	Nonadecane	269	Antimicrobial and Anticancer activity
15	2,6-Dihydroxybenzoic Acid	154	Antimicrobial and Anti-inflammatory
16	Sebacic Acid	202	Antimicrobial activity
17	Methyl 9,12-Epithiostearate	328	Antimicrobial activity
18	1-Isobenzofuranol	136	Antimicrobial activity
19	1h-Azepine-1-Carboxylic Acid	171	Antioxidant and Antimicrobial activity
20	Methyl Ester	298	Antimicrobial activity
21	9-Nonamethylpentasiloxane	342	Antimicrobial activity
22	D-Xylitol	152	Antimicrobial activity
23	Beta.-Sitosterol	414	Antimicrobial, Anti-inflammatory, Antidiabetic and Anticancer activity
24	Diethyl Docosanedioate	398	Antibacterial activity

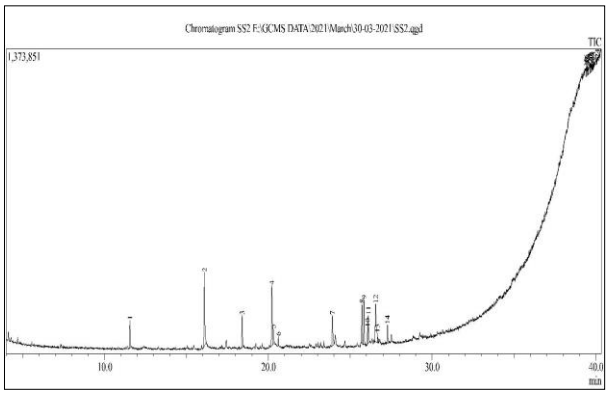


Fig 6 GCMS analysis of PVJ1

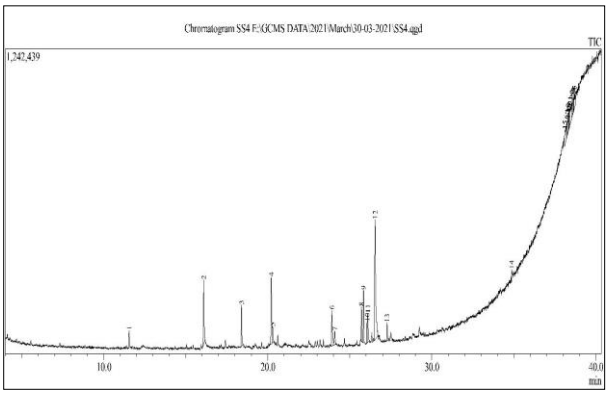


Fig 7 GCMS analysis of PVJ5

Table 8 GCMS analysis of PVJ5

S. No.	Phytochemicals	Molecular weight	Pharmacological actions
1	Tetradecane	198	Antimicrobial activity
2	2,4-Di-tert-butylphenol	206	Fungicidal activity
3	Hexadecane	226	Antimicrobial activity.
4	Cyclohexane	84	Antibacterial activity
5	Octadecane	254	Antibacterial activity
6	Cyclohexadecane	224	Antimicrobial activity
7	7,9-Di-tert-butyl-1-oxaspiro	276	Antimicrobial activity, antioxidant
8	2,5-cyclohexadien-1	94	Antibacterial activity
9	Hexadecanoic acid	256	Anti-inflammatory, antioxidant, Anticancer activities
10	Benzenepropanoic acid	164	Antimicrobial activity
11	1,2-Benzenedicarboxylic acid	276	Antifungal, antifouling and antidiarrhoeal activities
12	9-Oximino-3,6-dichloro fluorene	235	Anticancer, antimicrobial activity
13	Phosphonic acid	330	Herbicidal activity, antimalarial activity
14	Heptadecanoic acid	270	Antimicrobial activity, wound healing and antioxidant
15	Arabinitol	152	Anticancer activities
16	Sebacic acid	202	Antimicrobial activity
17	20- Icosa methyl cyclo decasiloxane	741	Antimicrobial activity
18	2,6-Lutidine 3,5-dichloro-4-dodecylthio-	375	Antibacterial activity

Plant roots secretes different kinds range of organic compounds in the rhizospheric region of soil that act as food source for microbes which leads to the increase microorganism density and other activities in the rhizospheric region compared to rhizoplane soil. The important activities of the microorganisms in the rhizosphere are (i) solubilization of inorganic P and organic P sources into the soluble P form (ii) nitrogen fixation (iii) phytohormone production (iv) ACC deaminase activity (v) siderophore production (vi) antipathogenic activity etc. Similarly inorganic phosphate solubilizing bacteria are largely available in the plant rhizosphere soil than in rhizoplane soil. In the present study 86 strains were isolated from the collected rhizosphere soil samples of *Tephrosia purpurea* (Linn.) plants from the different locations in Tamil Nadu. Phosphate solubilizing bacteria can be screened by a plate assay method using Pikovskaya medium. The bacteria will grow on this medium and form a clear zone around the colony [11-15]. These bacteria can able to convert the tricalcium phosphate which is present in the medium from insoluble to soluble forms [16]. Based on the solubilization zone around the bacterial colonies in our study 10 PSB were selected, characterized and screened for phosphate solubilization. PSB strains were isolated using the Pikovskaya's medium based on the formation of halo zone around the microorganisms [12]. The clear or halo zone was formed due to the solubilization of insoluble phosphates by bacteria isolated from different sources [18]. Phosphate solubilizing bacteria can able to convert unavailable P into available P for the dissolution and absorption by plants. Based on the above characteristics PSBs can be of two classes: (i) PSB produces organic acids to solubilize P compounds and (ii) P-mineralizing can be done by the microbes through the production of phosphatase enzymes. On the application of these PSB in soil decreases the pH of the soil [19]. The potential of phosphate solubilization of the PSBs is by their capacity to produce and release of acid metabolites by its hydroxyl and carboxyl groups which chelate the cations that bind to phosphate which helps in the conversion of insoluble phosphate into soluble form [8]. Based on the previous reports we have selected 10 isolates (PVJ1 to PVJ 10) for the studies of morphological and cultural characteristics and phosphate soubilization efficiency. The two isolates PVJ1 and PVJ 5 which shows high efficiency in phosphate solubilization than the other isolates by exhibiting highest zone formation of 33.6 mm and 41.6 mm were taken for further study. On the basis of biochemical studies the PVJ 1 was identified as *Bacillus* sp and PVJ 5 as *Bacillus* sp [20]. Phosphate solubilizing activity for the strains PVJ 1 and PVJ 5 were performed by determining the pH of broth inoculated with PVJ 1 and PVJ 5 for the period of 7 days of incubation. Phosphate solubilization potential of the strains is due to their ability to reduce pH of the surroundings by releasing organic acids or protons or by chelation [21]. In previous study reported that on incubation of the isolates the initial pH of the PKV broth was 7.0 which on further incubation up to 7 days reduce to 3.2 [5]. But in the current study the isolates PVJ1 and PVJ5 shows a greater reduction in pH from7.0 to 2.5 and 2.3 respectively in the period of 7 days of incubation. Many of the isolates which will not produce any visible halo zones on agar plate could also solubilize various types of insoluble inorganic phosphate in liquid medium. It may be due to various diffusion rates of different organic acids secreted by an organism. Therefore, phosphate solubilizing bacteria were further screened in NBRIP broth medium to evaluate their phosphate solubilizing efficiency. NBRIP medium contains bromophenol blue which changes its colour due to the decrease in pH of the medium. Hence, phosphate solubilizing efficiency of microorganisms can be easily screened based on visual observation [25]. In our study also the isolates PVJ1 and PVJ5 were further qualitatively screened in NBRIP medium which is supplemented with 0.1% bromothymol blue [22] to evaluate their phosphate solubilizing efficiency. The bromothymol blue which added as a pH indicator dye in the NBRIP medium changes its colour from blue to yellow around the colonies due to the reduction in pH by the isolates. By this the efficiency of solubilization of phosphate were easily screened by the visual observation from blue to yellow colour around the isolated colonies [23]. The efficient strains PVJ1 and PVJ5 are further studied by FITR and GCMS analysis. In FITR analysis (Table 5) of PVJI shows 9 peak values from 3447.97 cm^{-1} to 649.92 cm^{-1} which the bond varies from Alcoholic functional group followed by Alkane and Aromatic compound. Carboxylic acid peaks before the

Aliphatic and Halo compound with the Strong and Broad intensity whereas PVJ5 shows only 4 peak values from 3435.01 cm^{-1} to 652.04 cm^{-1} which the bond varies from Alcoholic followed by Strong intensity aromatic compound followed by the Halo compound of medium bond**.** Chromatogram GC-MS analysis of PVJ1 showed the presence of Twenty -four major peaks and the components corresponding to the peaks were determined as follows. The peaks that determined to be Dodecane and Tetradecane with the molecular weight of 170 and 198 which these compounds possess antimicrobial activity. The third compound 2,4-Di-Tert-Butylphenol which has Fungicidal activity with mass number of 206 followed by Hexadecane and Cyclopentane in which Hexadecane has the high molecular weight of 226 than Cyclopentane 70 but both has antimicrobial activity in addition Cyclopentane also has anticancer activities. The other compounds Octadecane, 2,5-Cyclohexadien-1 and Diethyl Docosanedioate has the antibacterial activity whereas Benzenepropanoic Acid, 1-Acetyl-4 –Piperazine, Sebacic Acid, Methyl 9,12-Epithiostearate, 1-Isobenzofuranol, Methyl Ester, 9-Nonamethylpentasiloxane and D-Xylitol possess the antimicrobial activity. P-Octylacetophenone has the anti-inflammatory activity. The other compound 7,9-Di-Tert-Butyl-1-Oxaspiro act as antioxidant and an antimicrobial agent. One of important compound is the Hexadecanoic Acid has multi pharmacological activities such as an antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Antiandrogenic, Heamolytic, Anti-inflammatory compound. 1, 2-Benzenedicarboxylic Acid possesses Antifungal, Antidiarrhoeal and Antifouling activities. Nonadecane has Antimicrobial and Anticancer activity. Antimicrobial and Anti-inflammatory activity are present in 2,6-Dihydroxybenzoic Acid. 1h-Azepine-1-Carboxylic Acid act as Antioxidant and Antimicrobial agent. Beta.-Sitosterol which has the highest molecular weight of 414 which has the Antimicrobial, Anti-inflammatory, Antidiabetic and Anticancer activity. Chromatogram GC-MS analysis of the methanol extract of PVJ5 showed the presence of Eighteen major peaks and the components corresponding to the peaks were determined as follows. The peaks that determined the compounds Tetradecane, Hexadecane, Cyclohexadecane, Benzenepropanoic acid, Sebacic acid, 20-Icosamethylcyclodecasiloxane show the antimicrobial activity. 2, 4-Di-tert-butylphenol has the Fungicidal activity whereas the other compounds Cyclohexane, Octadecane, 2, 5-cyclohexadien-1 and 2,6-Lutidine 3,5-dichloro-4-dodecylthio- determine to has the antibacterial activity. 7, 9-Di-tert-butyl-1-oxaspiro exhibit to have both antioxidant and antimicrobial activity. 1, 2-Benzenedicarboxylic acid has the ability for antifungal, antifouling and antidiarrhoeal activities**.** 9-Oximino-3, 6-dichloro fluorine has both anticancer and antimicrobial activity [26-27]. Phosphonic acids determine to have both herbicidal activity and antimalarial activity whereas Heptadecanoic acid exhibit to be an antioxidant, Wound healing compound and has antimicrobial activity. Arabinitol involved in anticancer activities. The phytochemical compounds such as Benzenepropanoic acid (BPA), Benzenedicarboxylic acid (BDA), Phosphonic acids and Sebacic acid produced by both the bacteria also involved in the mechanism of phosphate solubilization by chelating the iron present in the medium in the form of calcium phosphate and ferrous phosphate thereby resulting in the reduction the pH of the medium and formation of zone. These compounds exhibit to have antimicrobial activity which protects the plants from soil and plant pathogens. The present study concludes that when on application of these efficient strains PVJ1 and PVJ5 from the rhizosphere soil samples of *Tephrosia purpurea* (Linn.) a green manure crop as a biofertilizer gives good growth and yield to the agricultural crops due to its dual role of its high efficiency in phosphate solubilization and the benefits of plant and bacterial compounds effectiveness.

CONCLUSION

The current study reveals that the isolated effective strains of JPV 1 (*Bacillus* sp) and JPV 5 (*Bacillus* sp) from the rhizosphere soil of *Tephrosia purpurea* (Linn.) plant shown to have highest solubilization potential which can be used as a bioinoculant for the effective growth of plants due to the additional benefit of the isolates as the plant itself is a green manure and the phytochemical compounds of both the plant and the strain exhibit to have antimicrobial activity against plant and soil pathogens. Hence, we conclude that the application of these strains as a biofertilizer increases soil fertility and enhance the agriculture productivity.

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