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Molecular Identification, Compatibility and Shelf Life of Zinc and Silica Solubilizing Bacteria from Paddy Grown Soils

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ABSTRACT

The study was conducted during January to October, 2019 at Advanced Post Graduate Centre, Acharya N.G. Ranga Agricultural University, Guntur, Andhra Pradesh, India to characterize and their compatibility and shelf-life of zinc and silica solubilizing bacteria, isolates from paddy soil. Thirty-two (32) zinc solubilising bacteria and 28 silica solubilising bacteria were isolated from paddy soil samples of 20 villages in Kurnool, Prakasam, Guntur and Anantapur districts of Andhra Pradesh, India. Two efficient zinc solubilizing bacteria were identified as *Pseudomonas knackmussii* B13 with 99.80% similarity (ZnKJJ-4) and *Pseudomonas aeruginosa* strain FQM with 99.66% similarity (ZnPGG-1); two efficient silica solubilizing bacteria were as *Bacillus mucilaginosus* strain CGMCC 1.2326 with 99.95% similarity (SiKPP-1) and *Bacillus megaterium* PSB1 with 99.92% similarity (SiPYY-3) by using 16S rRNA phylogenetic study, plant growth promoting properties and biochemical characteristics. The sequenced isolates were submitted to the NCBI-GenBank, and obtained the accession numbers NR121733.1 (ZnKJJ-4), MF144507.1 (ZnPGG-1), FJ009518.1 (SiKPP-1) and MW020222.1 (SiPYY-3). Shelf-life studies using different carrier materials (Lignite, peat and charcoal powder) in the incubator at 25°C revealed that Zinc solubilizing bacterial isolates survived better in lignite whereas Silica solubilizing bacterial isolates persisted in charcoal powder. Compatibility of the isolates was verified in the form of perpendicular streaks at the interaction zone in nutrient agar medium after 48–72 h at 28°C prior to evaluation in direct sown paddy crop.

KEYWORDS: Paddy, zinc and silica solubilizing bacterial consortia

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Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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1. INTRODUCTION

Rice (*Oryza sativa* L.) is one of the vital staple foods for more than 50% of the world's population providing major source of the food energy. It is cultivated in 114 countries across the world in an area of 192 mha with annual production of 546.2 mt. In plants, zinc plays a crucial role as a structural constituent or regulatory co-factor of a wide range of different enzymes and proteins in many significant biochemical pathways and these are mainly concerned with: carbohydrate metabolism, both in photosynthesis and in the conversion of sugars to starch, protein metabolism, auxin (growth regulator) metabolism, pollen formation, maintenance of the integrity of biological membranes, resistance to infection by certain pathogens (Manasa et al., 2019). In the recent years importance of Zinc solubilizing bacteria (ZSB) has increased as probable candidates for improving bioavailable fraction of Zn to host plant for enhancing the crop growth, yield and quality without affecting the environment. Alternatively, several microorganisms, especially those associated with roots, have the ability to increase plant growth and productivity by enhancing the supply of mineral nutrients of low mobility in the soil like P, Zn and Cu (Thompson, 1996). Zinc is one of the trace elements, though required in small quantity, has dramatic growth-promoting effect as it plays a huge role in several processes related to enzymatic functions, protein synthesis, and metabolic path¬ways (Upadhayay et al., 2022a, b, c; Younas et al., 2023). The ecological role of ZSB is of paramount importance in maintaining soil fertility and nutrient cycling, as they contribute to the natural process of nutrient mobilization and promote plant-microbe interac¬tions that are essential for healthy plant growth (Sindhu et al., 2019; Haroon et al., 2022). Rice is a highly Si accumulating plant that shows a positive response to Si fertilizer regarding productivity (Singh et al., 2005). Silica is a beneficial plant nutrient with a vital role in maintaining plant growth and enhancing tolerance to both biotic and abiotic stresses (Mositofa et al., 2021; Etesami et al., 2018). The polymeric insoluble silica present in soils is solubilised during weathering to release monosilicic acid into the soil which is the bioavailable form of silicon absorbed by plants. Although silica (Si) is not considered as an essential element for higher plants, it has been proven to be beneficial for the well growth and development of many plant species, particularly tropical graminaceous plants such as rice (Liang et al., 2007). Adequate Si supply increased rice grain yield by enhancing the number of panicles per plant as well as the number of spikelets and the percentage of filled grains (Ma et al., 1989, Detmann et al., 2012). Though paddy is the greatest accumulator of Si up to 10% of its dry weight, it can't absorb. Continuous intensive rice cultivation and regular removal of rice straw every cropping season reduce the plant-available nutrient pools, especially Si (Li et al., 2019, Haynes et al., 2014). Synergistic action of *Pseudomonas* spp. and *Bacillus* spp. together in rhizosphere was reported by Ansari and Ahmad (2019), Qessaoui et al. (2019), Wei et al., 2019. Prolonged storage drastically reduced the efficiency of biofertilizers (O'Callaghan, 2016) and suitable carrier materials sustained their shelf life (Tabassam et al., 2015). Based on plate count method, 10% Trocalcium phosphate was found to be optimum to be used as an anticaking agent for biofertilizer containing *Pseudomonas*, *Rhizobium* and *Azospirillum*, respectively (Matura et al., 2021). In view of the importance of zinc and silica solubilizing bacteria, isolates from paddy grown soils of Andhra Pradesh were characterized and their compatibility and shelf-life studies were conducted during the present investigation.

2. MATERIALS AND METHODS

The study was conducted during January, 2019 to October, 2019 at Advanced Post Graduate Centre, Acharya N.G. Ranga Agricultural University, Guntur. The studies on molecular characterizationof efficient zinc and silica solubilizing bacterial isolates viz., ZnKJJ-4 (Zinc solubilizing isolate from soil sample–4 of Kurnool Dist., Jupadubunglow village and Mandal), ZnPGG-1 (Zinc isolate from soil sample–1 of Prakasam Dist., Giddaluru village and Mandal), SiKPP-1(Silica isolate from Kurnool Dist., Pamulapaduvillage and Mandal soil sample–1 (SiKPP-1) and SiPYY-3 Silica isolate from Prakasam Dist., Yerragondapalem village and Mandal soil sample–3 (SiPYY-3)} from paddy grown soilsand their compatibility and shelf-life studies were conducted.

2.1. Molecular identification of zinc and silica solubilizing bacterial isolates

The total genomic DNA of each isolate was extracted using a commercial DNA isolation kit (Hi Media) and quantified by recording the absorbance at 260 nm wavelength using a UV/VIS spectrophotometer and nanodrop. DNA was stored at-20°C for further use. The 16S rRNA gene of the target bacterial isolates was amplified by using universal eubacteria primers (Heddi et al., 1999). These were custom synthesized by Barcode Biosciences, Pvt. Ltd, India. Base sequences of 16S rRNA gene primerswere consisted of 27 forward primers and 1492 reverse primers (Kumar et al., 2024). The PCR amplification was carried out in 0.2 ml PCR tubes with 25 µl reaction volume consisting 2.5 µl of 10x buffer, 1.5 µl of 25 mM $MgCl_2$, 2.0 µl of dNTPs mix (10 mM each), 1.0 µl each of primers (20 p mol), 2.0 µl of DNA (10 ng μl^{-1}) plus distilled water. The reaction mixture was vortexed and centrifuged in a refrigerated centrifuge (Eppendorf). Amplifications were performed using a thermal cycler (Eppendorf PCR systems) with initial

denaturation at 94°C for 5 min. followed by denaturation at 94°C for 45 sec., annealing at 53°C for 45 sec and elongation at 72°C for 30 sec. The thermal cycler was programmed for 30 cycles with one cycle of initial denaturation and steps 2–4 were repeated 30 times and a final extension at 72°C for 30 sec using the fastest ramp time between the temperature transitions.

The amplified PCR products were resolved by electrophoresis using 1% agarose gel in 1x Tris-acetate EDTA buffer (2 M Tris base, 57.10 ml acetic acid and 0.5 M EDTA, pH 8.0, 50 x). Agarose gel was mixed with ethidium bromide (0.5 µg ml^{-1}) before pouring on the tray. The gel was run using 1 kb DNA ladder (Bangalore Genei) as markerat 120 V for 45 min. The ethidium bromide stained gel was viewed and the image was captured using a gel documentation system (Biorad systems).

PCR products of the 16S rRNA gene of four efficient bacterial isolates obtained through amplification with specific primers were freeze dried in a lyophilizer and sent for custom sequencing using the same upstream and downstream primers used for the amplification of 16S rRNA gene (Barcode Biosciences, Pvt. Ltd, India). The 16S rRNA sequences of different bacterial isolates were BLAST (Basic local alignment search tool) searched against the sequences of 16S rRNA of bacterial isolates available in the NCBI Genbank Nucleotide Database (www.ncbi.nlm.nih.gov). Based on maximum identity score sequences were selected and aligned using multiple alignment software program Clustal W (http://clustalw.genome.jp/). Distance matrix was generated using the RDP database and the phylogenetic tree was constructed using MEGA X (Tamura et al., 2007) (http://megasoftware.net).

2.2. Screening of different carrier materials for formulating the efficient zinc and silica solubilizing isolates

Different carrier materials viz., peat (collected from Nilgiri hills, Coimbatore, Tamil Nadu), lignite (from Biofertilizers unit, Agriculture Research Station, Amaravathi, ANGRAU, Guntur) and charcoal powder (Quality traders, Guntur) were collected and screened against zinc and silica solubilizing isolates for improved shelf life. They were sterilized in tyndalization process in an autoclave at 15lb psi (121°C) for 20 min three times on succeeding days. Prior to preparation of bioformulation, physico-chemical properties including moisture content and pH were recorded (Table 1). Each carrier material was milled to pass through 100–200 µm mesh sieve by manual sieving, pH of the carrier material was neutralized by adding calcium carbonate. Sterilization of the carrier material was done at 121°C for 15 min. at 15lb pressure before inoculation.

The selected isolates were multiplied in large quantities in appropriate culture broth by incubating at 28±2°C in an incubator shaker till they attained log phase with a cell load of 1×10^9 cfu ml^{-1} and were used for carrier based microbial inoculants preparation (Kaljeet et al., 2011). The individual carrier materials were powdered and the pH was brought to neutral by adding $CaCO_3$, sterilized at 15 psi for three successive days and allowed to cool overnight and then mixed with the log phase culture (1×10^9 cfu ml^{-1}) of the selected plant growth promoting microbial inoculants in separate quantities in shallow trays. The optimum moisture content was adjusted to (30–40%) prior to preparation, followed by curing in shallow trays for 24 h under aseptic conditions and then packed in high density opaque polythene bag (12 g) at the rate of 500 g bag^{-1} and sealed. Individual microbial consortia were then prepared by mixing with peat, lignite and charcoal powder in 1:3 volumes of each culture broth of microbial consortia with sterile carrier materials. The populations of individual zinc and silica solubilizing bacterial inoculant carriers were assessed at monthly intervals upto 6 months. Twelve treatments viz., T_1: Peat+ZnKJJ-4; T_2: Peat+ZnPGG-1; T_3: Peat+SiKPP-1; T_4: Peat+SiPYY-3; T_5: Lignite+ZnKJJ-4; T_6: Lignite+ZnPGG-1; T_7: Lignite+SiKPP-1; T_8: Lignite+SiPYY-3; T_9: Charcoal powder+ZnKJJ-4; T_{10}: Charcoal powder+ZnPGG-1; T_{11}: Charcoal powder+SiKPP-1; T_{12}: Charcoal powder+SiPYY-3 were imposed for carrier materials.

Table 1: Physico-chemical properties of carrier materials

Parameters	Peat	Lignite	Charcoal powder
pH	5.4	6.1	7.5
Water holding capacity	140	192	190
Moisture (%)	45	49	42
Bulk density (%)	1.02	1.06	0.50

2.3. Compatibility test

The compatibility of the isolates was tested to develop the efficient microbial consortia/inoculants. The single bacterial strain was streaked as a straight line in the centre of the tryptic soy agar plate. Cultures to be tested were streaked perpendicularly across the initial culture and incubated at 28°C for 48–72 h. Lack of microbial growth (zone of inhibition) at the intersections was indicative of the antagonistic activity of the cultures but the colonies growing in the proximity were compatible with each other. Compatibility study was done by streaking dual inoculants viz., i) ZnKJJ-4+ZnPGG-1; ii) SiKPP-1+SiPYY-3; iii) ZnKJJ-4+SiKPP-1; iv) ZnPGG-1+SiPYY-3; v) ZnKJJ-4 and ZnPGG-1+SiKPP-1 and SiPYY-3 on solidified nutrient agar medium.

2.4. Development of microbial consortia/inoculants

Isolates were cultured individually, using specific media then

mixed and grown together in a conical flask at 10 ml isolate^{-1}. The population of each bacterial isolate in the consortia was monitored regularly in the conical flask and also in the soil to which they are applied for their screening against the host crop under greenhouse conditions.

3. RESULTS AND DISCUSSION

3.1. Molecular identification of efficient zinc and silica solubilizing bacterial isolates

Molecular identification by 16S rRNA gene sequencing was taken up for four efficient isolates viz., ZnKJJ-4, ZnPGG-1, SiKPP-1 and SiPYY-3. Amplicon product is showed in Figure 1. Sequencing of Zinc solubilizing bacterial strains i.e., ZnKJJ-4 and ZnPGG-1 by using Sanger method and blasting with consensus sequence (NCBI-BLASTn) showed similarity with *Pseudomonas knackmussii* strain B13 (99.80% similarity) and *Pseudomonas aeruginosa* strain FQM (99.66 % similarity). Sequencing of Silica solubilizing bacterial strains i.e., SiKPP-1 and SiPYY-3 by using Sanger method and blasting with consensus sequence (NCBI-BLASTn) showed similarity with *Bacillus mucilaginosus* strain CGMCC 1.2326 (99.95% similarity) and Bacillus

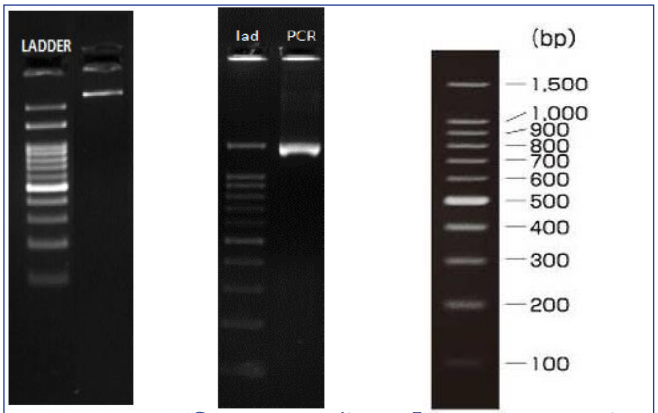

Figure 1: Amplification product of 16S rRNA, gDNA and ladder specification

megaterium PSB1 (99.92% similarity), respectively (Table 2). Phylogenetic tree and timeline tree were constructed using MEGA-X software (Figure 1 to 5). All the isolates sequenced were submitted to the NCBI-GenBank, online

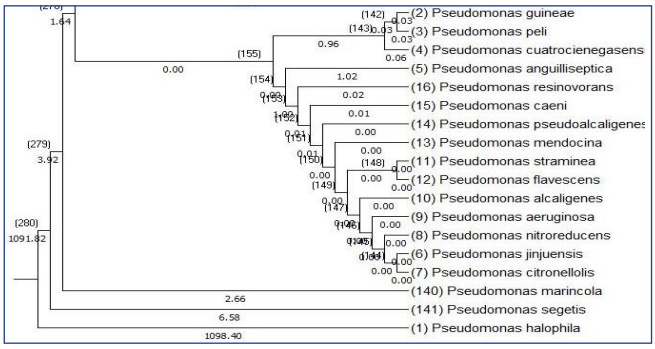

Figure 2: Phylogenetic relationship of efficient zinc solubilizing bacteria isolated from direct sown paddy (*Oryza sativa* L.) growing areas soils of Andhra Pradesh, Kurnool, Guntur, Anantapur and Prakasam districts

repository/library of sequences, and the isolates were given the accession numbers. 16S rRNA region in the small subunit of the ribosome was a highly conserved and ubiquitous region present in bacteria and archaea. This highly conservation region made it possible to construct universal primers and provides a deep understanding of the isolates at species level using a public database search (NCBI-BLAST). It was immensely essential to note that not all the domains of 16S rRNA were capable of differentiating between species. Since this region was well known, construction of phylogenetic tree was easy

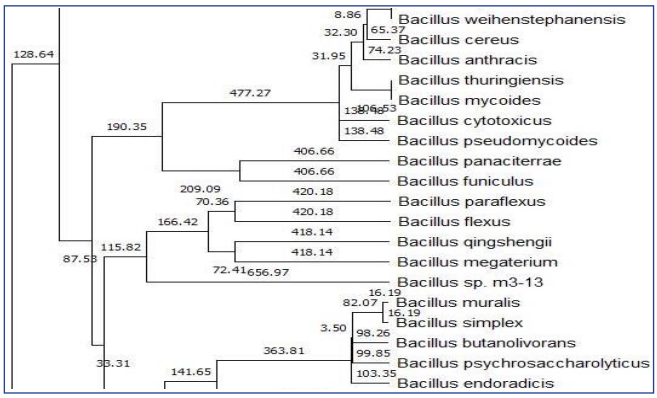

Figure 3: Phylogenetic relationship of efficient silica solubilizing bacteria isolated from direct sown paddy (*Oryza sativa* L.) growing areas soils of Andhra Pradesh, Kurnool, Guntur, Anantapur and Prakasam districts

Table 2: Molecular identification of zinc and silica solubilizing bacterial isolates from direct sown paddy growing areas of Andhra Pradesh

Organism	Isolate code	Species	Similarity of 16S rRNA gene sequence (%)	Accession
ZnSB	ZnKJJ-4	*Pseudomonas knackmussiistrain* B13	99.80%	NR121733.1
ZnSB	ZnPGG-1	*Pseudomonas aeruginosa* strain FQM	99.66%	MF144507.1
SiSB	SiKPP-1	*Bacillus mucilaginosus* strain CGMCC 1.2326	99.95%	FJ009518.1
SiSB	SiPYY-3	*Bacillus megaterium* PSB1	99.92%	MW020222.1

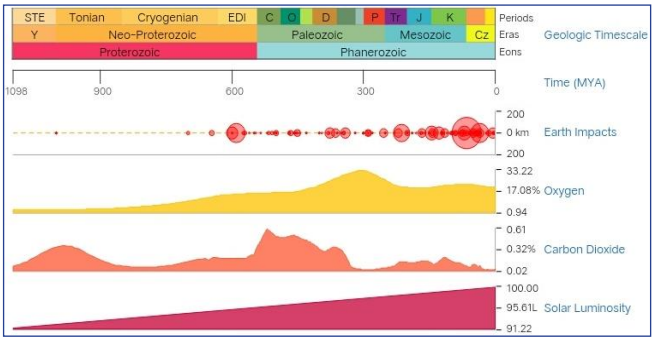

Figure 4: Timeline tree constructed using timetree in MEGA-X software for *Pseudomonas* sp.

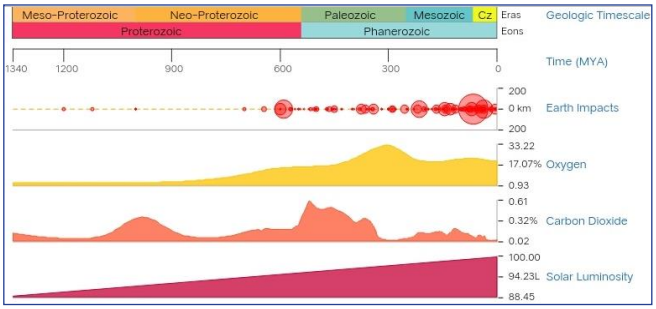

Figure 5: Timeline tree constructed using timetree in MEGA-X software for *Bacillus* sp.

with ample database. Isolates with >97% homology were grouped as similar species and below 97% homology were referred to as different species (Kim et al., 2014). However, deeper understanding of strain level could be made by using Average Nucleotide Identity (ANI) and DDH (DNA-DNA hybridization) techniques (Chan et al., 2012).

3.2. Compatibility tests of the efficient zinc and silica solubilizing microbial isolates to develop microbial consortia

Five bacterial isolates selected from *in planta* experiment were subjected to mutual compatibility test by cross streak method. No lysis was observed at the juncture of ZnKJJ-4+ ZnPGG-1, SiKPP-1+SiPYY-3, ZnKJJ-4+SiKPP-1, ZnPGG-1+SiPYY-3, and ZnKJJ-4 and ZnPGG-1+SiKPP-1 and SiPYY-3 combinations, which indicated the compatibility among the isolates (Plate 1).

Deepa and Mathew (2017) studied compatibility of fungi, bacteria and actinomycetes, prepared a consortium and found three of the five bacterial isolates EkRB-1, VSB-1 and TRB-1 were compatible. Choure and Dubey (2012) developed plant growth promoting microbial consortium based on interactions studies. Three strains viz., *Pseudomonas fluorescens* LPK2,

Streptomyces fredii KCC5 and *Azotobacter chroococcum* AZK2 did not inhibit each other. Spectrophotometric studies also showed that the individual growth of these strains was not affected in combined cultures, where strains were cultured together. Singh et al. (2013) developed and investigated microbial consortium activity on *Cicer arietium* against *Sclerotium rolfsii*. The organisms used in consortia preparation were *Pseudomonas aeruginosa* (PHU094), *Trichoderma harzianum* (THU0816), *Mesorhizobium* sp. (RL091). The reasons for the best action of microbial consortia compared to individual inoculation were activation of phenylpropanoid pathway, lignin deposition antioxidant mechanism.

3.3. Screening of different carrier materials for formulating the efficient carrier-based zinc and silica solubilizing isolates

Peat carrier: The population of ZnKJJ-4 and ZnPGG-1 in T_1 (Peat+ZnKJJ-4) and T_2 (Peat+ZnPGG-1) was 8.42 and 8.43 Log CFU g^{-1} carrier at zero day, respectively. On 15th day, population of ZnKJJ-4 and ZnPGG-1 significantly increased to 8.51 and 8.50 Log CFU g^{-1} carrier in T_1 (Peat+ZnKJJ-4) and T_2 (Peat+ZnPGG-1). There was significant increase in the population of ZnKJJ-4 and ZnPGG-1 upto the end of 2nd month (60th day) in T_1 (Peat+ZnKJJ-4) and T_2 (Peat+ZnPGG-1) (9.08 and 9.14 Log CFU g^{-1} carrier), respectively. After that ZnKJJ-4 and ZnPGG-1 population significantly decreased and on 90th day ZnKJJ-4 and ZnPGG-1 population in T_1 (Peat+ZnKJJ-4) and T_2 (Peat+ZnPGG-1) was 8.61 and 8.58 Log CFU g^{-1} carrier, respectively. At the end of the storage on 180th day ZnKJJ-4 and ZnPGG-1 population in T_1 (Peat+ZnKJJ-4) and T_2 (Peat+ZnPGG-1) was 6.06 and 6.04 Log CFU g^{-1} carrier, respectively (Table 3).

The population of SiKPP-1 and SiPYY-3 in T_3 (Peat+SiKPP-1) and T_4 (Peat+SiPYY-3) was 8.46 and 8.51 Log CFU g^{-1} carrier at zero day, respectively. On 15th day SiKPP-1 and SiPYY-3 population significantly increased to 8.51 and 8.52 Log CFU g^{-1} carrier in T_3 (Peat+SiKPP-1) and T_4 (Peat+SiPYY-3) respectively. There was significant increase in the population of SiKPP-1 and SiPYY-3 upto the end of 2nd month (60th day) in T_3 (Peat+SiKPP-1) and T_4 (Peat+SiPYY-3) was 9.01 and 9.08 Log CFU g^{-1} carrier. Eventually, SiKPP-1 and SiPYY-3 population significantly decreased and on 90th day SiKPP-1 and SiPYY-3 population in T_3 (Peat+SiKPP-1) and T_4 (Peat+SiPYY-3) was 8.52 and 8.57 Log CFU g^{-1} carrier. At the end of the storage, on 180th day SiKPP-1 and SiPYY-3 population in T_3 (Peat+SiKPP-1) and T_4 (Peat+SiPYY-3) was 6.02 and 6.04 Log CFU g^{-1} carrier respectively (Table 3).

Lignite: The population of ZnKJJ-4 and ZnPGG-1 in T_5 (Lignite+ZnKJJ-4) and T_6 (Lignite +ZnPGG-1) was 8.42 and 8.43 Log CFU g^{-1} carrierat zero day, respectively. On 15th day ZnKJJ-4 and ZnPGG-1 population significantly increased to 8.54 and 8.53 Log CFU g^{-1} carrier in T_5 (Lignite+ZnKJJ-4) and T_6 (Lignite+ZnPGG-1). There was significant increase in the population of ZnKJJ-4 and ZnPGG-1 upto the end of 2nd month (60th day) in T_5

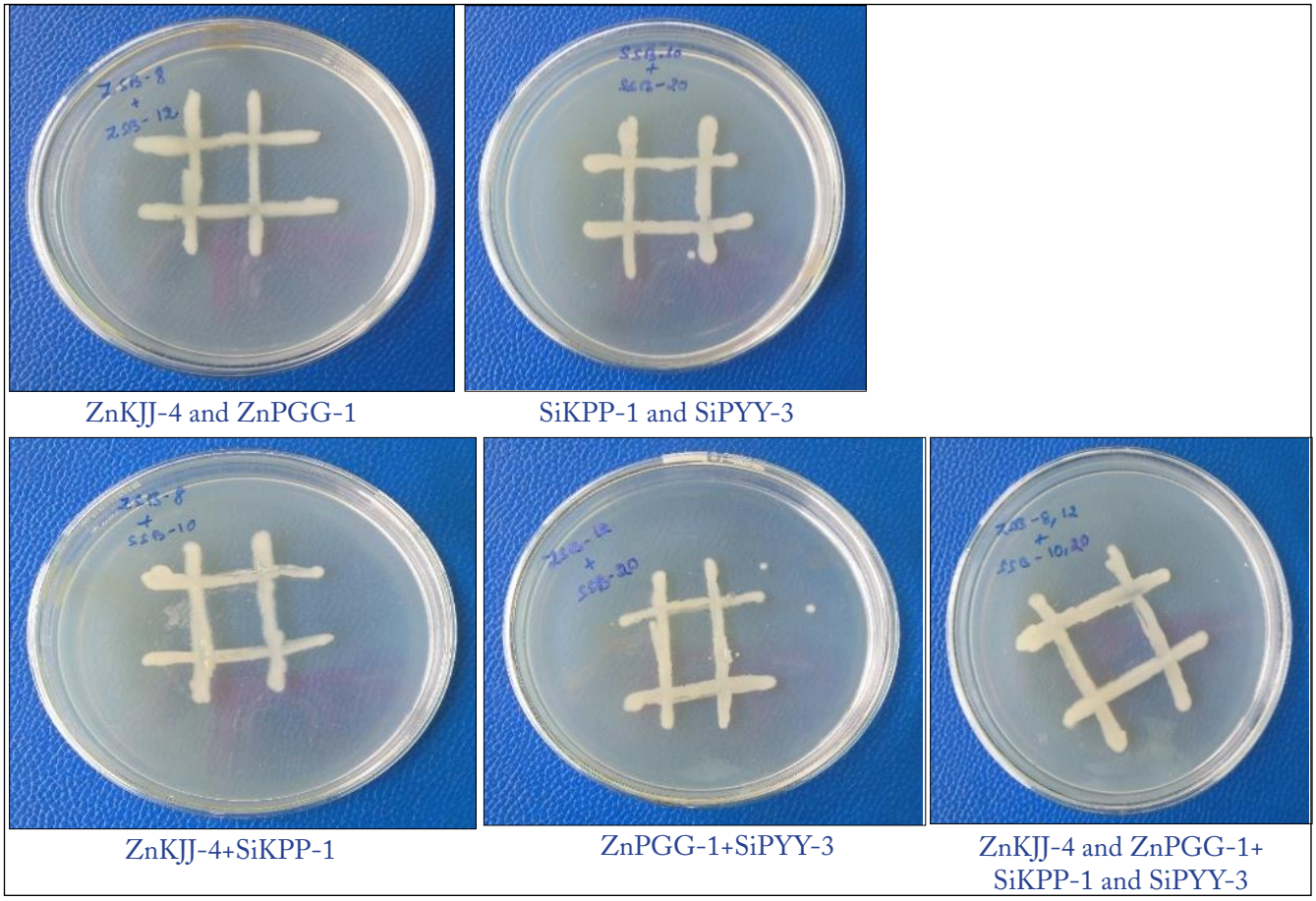

Plate 1: Compatibilty of zinc and silica solubilizing microorganisms

(Lignite+ZnKJJ-4) and T_6 (Lignite+ZnPGG-1) (9.15 and 9.16 Log CFU g^{-1} carrier), respectively. SubsequentlyZnKJJ-4 and ZnPGG-1 population significantly decreased and on 90th day ZnKJJ-4 and ZnPGG-1 population in T_5 (Lignite+ZnKJJ-4) and T_6 (Lignite+ZnPGG-1) was 8.63 and 8.64 Log CFU g^{-1} carrier, respectively. At the end of the storage on 180th day ZnKJJ-4 and ZnPGG-1 population in T_5 (Lignite+ZnKJJ-4) and T_6 (Lignite+ ZnPGG-1) was 6.18 and 6.25 Log CFU g^{-1} carrier (Table 3), respectively.

The population of SiKPP-1 and SiPYY-3 in T_7 (Lignite+SiKPP-1) and T_8 (Lignite+SiPYY-3) was 8.46 and 8.51 Log CFU g^{-1} carrier at zero day, respectively. On 15th day SiKPP-1 and SiPYY-3 population significantly increased to 8.52 and 8.53 Log CFU g^{-1} carrier in T_7 (Lignite+SiKPP-1) and T_8 (Lignite+SiPYY-3). There was significantly increase in the population of SiKPP-1 and SiPYY-3 upto the end of 2nd month (60th day) in T_7 (Lignite+SiKPP-1) and T_8 (Lignite+SiPYY-3) (9.11 and 9.14 Log CFU g^{-1} carrier) respectively. Subsequently SiKPP-1 and SiPYY-3 population significantly decreased and on 90th day SiKPP-1 and SiPYY-3 population in T_7 (Lignite+SiKPP-1) and T_8 (Lignite+SiPYY-3) was 8.58 and 8.59 Log CFU g^{-1} carrier respectively. At the end of the storage on 180th day SiKPP-1 and SiPYY-3 population in T_7 (Lignite+SiKPP-1) and T_8 (Lignite+SiPYY-3) was 6.06 and 6.08 Log CFU g^{-1} carrier respectively (Table 3).

Charcoal powder: The population of ZnKJJ-4 and ZnPGG-1 in T_9 (Charcoal powder+ZnKJJ-4) and T_{10} (Charcoal powder+ZnPGG-1) was 8.42 and 8.43 Log CFU g^{-1} carrierat zero day, respectively. On 15th day ZnKJJ-4 and ZnPGG-1 population significantly increased to 8.51 and 8.49 Log CFU g^{-1} carrier in T_9 (Charcoal powder+ZnKJJ-4) and T_{10} (Charcoal powder+ZnPGG-1). There was significant increase in the population of ZnKJJ-4 and ZnPGG-1 upto the end of 2nd month (60th day) in T_9 (Charcoal powder+ZnKJJ-4) and T_{10} (Charcoal powder+ZnPGG-1) (9.04 and 9.08 Log CFU g^{-1} carrier), respectively. ThereafterZnKJJ-4 and ZnPGG-1 population significantly decreased and on 90th day ZnKJJ-4 and ZnPGG-1 population in T_9 (Charcoal powder+ZnKJJ-4) and T_{10} (Charcoal powder+ZnPGG-1) was 8.59 and 8.58 Log CFU g^{-1} carrier. At the end of the storage on 180th day ZnKJJ-4 and ZnPGG-1 population in T_9 (Charcoal powder+ZnKJJ-4) and T_{10} (Charcoal powder+ZnPGG-1) was 6.02 and 6.01 Log CFU g^{-1} carrier, respectively (Table 3).

Table 3: Shelf life of ZnSB and SiSB in different carrier materials over a period of storage (Log CFU g^{-1} Soil)

Treatments	Zero day	15th day	30th day	60th day	90th day	120th day	150th day	180th day
T_1	8.42	8.51	8.54	9.08	8.61	8.46	7.54	6.06
T_2	8.43	8.50	8.52	9.14	8.58	8.46	7.42	6.04
T_3	8.46	8.51	8.55	9.01	8.52	8.32	7.31	6.02
T_4	8.51	8.52	8.54	9.08	8.57	8.39	7.29	6.04
T_5	8.42	8.54	8.68	9.15	8.63	8.54	7.56	6.18
T_6	8.43	8.53	8.58	9.16	8.64	8.51	7.65	6.25
T_7	8.46	8.52	8.59	9.11	8.58	8.38	7.35	6.06
T_8	8.51	8.53	8.56	9.14	8.59	8.42	7.31	6.08
T_9	8.42	8.51	8.52	9.04	8.58	8.43	7.3	6.02
T_{10}	8.43	8.49	8.50	9.08	8.42	8.42	7.32	6.01
T_{11}	8.46	8.54	8.62	9.19	8.61	8.42	7.38	6.15
T_{12}	8.51	8.55	8.58	9.20	8.62	8.49	7.52	6.18
SEm±	0.005	0.004	0.006	0.005	0.008	0.007	0.009	0.004
CD (p=0.05)	0.016	0.014	0.017	0.015	0.023	0.022	0.027	0.012
CV (%)	0.182	0.128	0.146	0.142	0.185	0.186	0.124	0.185

T_1: Peat+ZnKJJ-4; T_2: Peat+ZnPGG-1; T_3: Peat+SiKPP-1; T_4: Peat+SiPYY-3; T_5: Lignite+ZnKJJ-4; T_6: Lignite+ZnPGG-1; T_7: Lignite+SiKPP-1; T_8: Lignite+SiPYY-3; T_9: Charcoal powder+ZnKJJ-4; T_{10}: Charcoal powder+ZnPGG-1; T_{11}: Charcoal powder+SiKPP-1; T_{12}: Charcoal powder+SiPYY-3

The population of SiKPP-1 and SiPYY-3 in T_{11} (Charcoal powder+SiKPP-1) and T_{12} (Charcoal powder+SiPYY-3) was 8.46 and 8.51 Log CFU g^{-1} carrierat zero day, respectively. On 15th day SiKPP-1 and SiPYY-3 population significantly increased to 8.54 and 8.55 Log CFU g^{-1} carrier in T_{11} (Charcoal powder+SiKPP-1) and T_{12} (Charcoal powder +SiPYY-3). There was significant increase in the population of SiKPP-1 and SiPYY-3 population upto the end of 2nd month (60th day) in T_{11} (Charcoal powder+SiKPP-1) and T_{12} (Charcoal powder+SiPYY-3) (9.19 and 9.20 Log CFU g^{-1} carrier) respectively. Afterwards SiKPP-1 and SiPYY-3 population significantly decreased and on 90th day SiKPP-1 and SiPYY-3 population in T_{11} (Charcoal powder+SiKPP-1) and T_{12} (Charcoal powder+SiPYY-3) was 8.61 and 8.62 Log CFU g^{-1} carrier. At the end of the storage on 180th day SiKPP-1 and SiPYY-3 population in T_{11} (Charcoal powder+SiKPP-1) and T_{12} (Charcoal powder+SiPYY-3) was 6.15and 6.18 Log CFU g^{-1} carrier (Table 3).

Results revealed that lignite has the capability to give more shelf life compared to peat and charcoal powder for the zinc solubilizing bacterial (ZnSB) isolates and chosen as carrier material for both ZnKJJ-4 and ZnPGG-1 isolates. In case of silica solubilizing bacteria (SiSB) charcoal powder showed more viability for both SiKPP-1 and SiPYY-3 isolates, thus used as carrier material.

4. CONCLUSION

Efficient zinc and silica solubilizing bacteria were identified as Pseudomonas knackmussii B13 with 99.80% similarity (ZnKJJ-4), Pseudomonas aeruginosa strain FQM with 99.66% similarity (ZnPGG-1), Bacillus mucilaginosus strain CGMCC 1.2326–99.95% similarity (SiKPP-1) and Bacillus megaterium PSB1-99.92% similarity (SiPYY-3) by using 16S rRNA phylogenetic study. These bacteria confirmed their compatibility in dual cultures.

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