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Molecular Depiction of Vermicompost Associated Bacteria Possessed Agricultural Traits

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SA conceived the idea, designed the experiments, analysed and interpreted the results, and wrote manuscript. AN helped in the designing of experiments. FK and FA performed the experiments and wrote the first draft. WAB contributed in molecular depiction using bioinformatics tools. MFK and SA did formal analysis, interpreted results and manuscript review.

Key words
Vermicompost, Vermibacteria, Biochemical tests, Ribotyping, Azad Kashmir, *E. fetida*

ABSTRACT

Vermicomposting is an environmentally cordial process in which different organic wastes are converted into compost by earthworms. Vermicompost is the end product of vermicomposting which is a peat-like material, highly porous with maximum aeration and drainage capacity. It is rich in essential nutrients and beneficial microbes which are very useful for plant development and growth. The current research was conducted to isolate and identify some beneficial bacteria from the vermicompost which could be used as plant growth-promoting bacteria (PGPB) in the future to enhance crop production in Pakistan. Cattle dung, organic waste materials (egg shells, coconut peels, paper waste, raw vegetables, and fruits), and *Eisenia fetida* were used for vermicompost production. Vermicompost-associated bacteria were identified using microscopic techniques, various biochemical tests, cultural media, and ribotyping. Plant growth-promoting traits i.e. Indole acetic acid, siderophore, ammonia, hydrogen cyanide, and potassium hydroxide were also performed. Antibiogram analysis was also done to screen the multi-drug-resistant bacteria. Results revealed that the isolated vermicompost-associated bacteria were identified as *Achromobacter ruhlandii, Bacillus wiedmannii, Pseudomonas* sp., *Achromobacter xylosoxidans, Oceanobacillus oncorhynchi, Bacillus mycoides, Serratia nematodiphila, Serratia marcescens*, and *Paenibacillus dendritiformis* possessed plant growth promoting traits, non-pathogenic, and non-multi-drug resistant bacteria. It was concluded that vermicompost-associated bacteria could be used as a potential source of biofertilizers not only to enhance plant growth and development, and soil fertility, but also to be useful for the control of pests and pathogens for sustainable agriculture in Pakistan.

INTRODUCTION

Soil microorganisms i.e., bacteria, fungi, and actinomycetes involved in the soil formation, improving the soil fertility *via* degradation and detoxification of organic materials in soil, and maintain the ecological balance (Emperor *et al*., 2015; Zhu *et al*., 2017). Vermicomposting is an ecofriendly, bio-oxidative, and non-thermophilic organic decomposition technology to recycle the organic waste into fine granular product called vermicompost *via* the joint action of earthworms and microorganisms (Lazcano *et al*., 2008; Chitrapriya *et al*., 2013) (Supplementary Fig. 1). Various earthworm species

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Abbreviations
PGPB, plant growth-promoting bacteria; VAB, vermicompost associated bacteria; TMP, Trimethoprim; ATM, Aztreonam; K, Kanamycin, E, Erythromycin, S, Sulfonamide, TOB, Tobramycin; P, Penicillin; NOR, Norfloxacin, AMC, Amoxicillin and AMP, Ampicillin.

i.e., *Metaphire californica, Eudrilus eugeniae, Eisenia andrei, Perionyx excavates,* and *Eisenia fetida* were used for vermicomposting (Deka *et al*., 2011; Varma *et al*., 2016; Boruah *et al*., 2019; González-Moreno *et al*., 2022).

Earthworms ingest soil microbes, their population increases *via* passage through the intestinal track due to favorable environment, and excrete them along with nutrients in their vermicast (Huang *et al*., 2013; Pathma and Sakthivel, 2012). Several antibiotics and enzymes play an important role in the decomposition and degradation of organic molecules which were released by microbes in the gastrointestinal track of earthworms (Lazcano *et al*., 2008).

Vermicompost is not only rich in micro- and macronutrients, enzymes, plant growth hormones, plant growth regulators, and antibiotics (Ravindran *et al*., 2016; Shafique *et al*., 2021) but also improves the plant growth, plant nutrition and productivity, soil fertility, soil structure as well as growth of soil beneficial microbes (Pathma and Sakthivel, 2012; Zhu *et al*., 2017) (Supplementary Fig. 1). In addition, vermicompost improves soil aeration, moisture contents, pH, electrical conductivity, and water holding capacity (Shafique *et al*., 2021). Vermicompost suppresses plant diseases and increased microbial population like phosphate solubilizers, nitrogen fixers, Indole acetic acid producers, siderophore producers, which could significantly enhance the vegetative plant growth of ornamental plants (Shafique *et al*., 2021; Chauhan and Singh, 2015). Previous literature reported the presence of bacteria in vermicompost produced *via L. mauritii, P. excavatus* and *E. eugeniae* (Upadhyay *et al*., 2015; Selvi and Koilraj, 2015; Begum and Bora, 2018). Devi *et al.* (2009) and Pathma and Sakthivel (2012) illustrated the presence of Proteobacteria, Bacteroidetes Planctomycetes, Actinobacteria, and Firmicutes in vermicompost (Yasir *et al*., 2009a).

In view of above the objectives of the current research were to isolate, characterize, and identify vermicompost associated bacteria from vermicompost produced via *E. fetida*, to explore the agricultural traits and to screen the prevalence of antibiotic resistance among vermicompost associated bacteria. No research has been done before on the beneficial vermi-bacterium isolation and identification from vermicompost in Pakistan. Hopefully, this identified bacterial isolates could be used as microbial biofertilizer to enhance the seed germination and plant growth of various crops in future.

MATERIALS AND METHODS

Chemicals used

Nutrient broth (lennox), Luria Bertani (LB) broth (lennox), nutrient agar medium (sigma-aldrich), hydrogen peroxide, oxidase reagent, gram staining kit (merck), skim milk agar (neogen), Wattman No.1 disc, MacConkey agar (sigma-aldrich), mannitol salt agar (oxoid), Nessler's reagent (sigma-aldrich), Kovac's reagent, phenol, 0.5% Picric acid (sigma-aldrich), King's B medium (Sigma), 2% sodium carbonate (merck), dilute Iodine, 3% KOH starch (sigma-aldrich) and bacteriological Peptone (oxoid). The antibiotics used were trimethoprim (TMP 5µg), aztreonam (ATM 30µg), kanamycin (K 30µg), erythromycin (E 15µg), sulfonamide (S 300µg), tobramycin (TOB 10µg), penicillin (P 10µg), norfloxacin (NOR 10µg), amoxicillin (AMC 30µg) and ampicillin (AMP 25µg). All these antibiotics were taken from Oxide company.

Glassware and types of equipment used

Steam sterilizer (autoclave), Laminar flow (ESO Prod Model; EQU/03-EHC; Serial # 2000-0052), 37ºC incubator (MMM group Medcenter Enrich tungsten GmbH), Analytical balance (SARTORIUS GMBM GOTTINGEN, Germany), sterile distilled water, sterile bottles, sterile dissecting pins, dissecting box, dissecting board, gloves, 37ºC shaker (Irmeco GmbH, Germany), digital weighing machine (Jeweler Precision Balance Model: DH-V600A), 70% Ethanol, 500 ml beakers, 250 ml conical flasks, test tubes, petri plates, L shaped glass rod, microscope, bacteriological wire loop, micropipette, glass slides, glycerol, coverslips, spirit lamp and toothpicks.

Isolation, enumeration, and purification of vermi-bacteria

Vermicompost associated bacteria (VAB) were isolated from the vermicompost collected from Vermi-tech Unit, Department of Zoology, The University of Azad Jammu and Kashmir, Muzaffarabad. Vermicompost was prepared using cow dung, raw vegetables and fruits, coconut peel, wheat straw and rice straw, egg shells and waste papers. *Eisenia fetida* was used for the vermicomposting (Shafique *et al*., 2021). Hundred mg vermicompost was added in 200 ml of distilled water, mixture was shaken for 20 min, and kept at room temperature for two days. After incubation, serial dilution method was used as illustrated by Somasegaran and Hoben (2012) for the isolation of vermicompost associated bacteria. Nutrient broth medium was used for bacterial culturing. After incubation, serial dilution 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} were made and again incubated for 24 h at room temperature to pick the single colony of VAB. After incubation, 10 µl sample was spread on nutrient agar medium and incubated at 37°C for overnight. Next day, several colonies were observed in the case of 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} diluted samples. From these plates, twenty bacterial isolates were taken in nutrient broth medium and incubated for 24 h at 37°C. After incubation,

14 VAB isolates (VAB-1 to VAB-14) were purified after sub-culturing and then kept at -20 °C in the form of 60% glycerol.

Morphological and biochemical characteristics of VAB

For the identification of VAB different techniques and methods i.e. microscopic (Gram staining), cultural techniques (nutrient agar medium, MacConkey agar), biochemical tests (Plant growth promoting traits like production of Indole acetic acid, siderophore, ammonia, hydrogen cyanide, potassium hydroxide), hydrolytic enzymes production like catalase, oxidase, amylase, protease, and lipase, and hemolytic test were applied (Okon *et al*., 1977; Dawwam *et al*., 2013; Kumar *et al*., 2015). The different colors, size, elevations, textures, shapes, and edges of vermicompost associated bacteria were recorded.

Molecular characterization

Genomic DNA was extracted from bacterial isolates using chloroform: Isoamyl alcohol method with slight modifications. The isolates were grown in Luria broth medium for 24 h at 37 °C. After incubation, medium was centrifuged at 10,000 rpm for 5 min to harvest cells in pellet form. The pellet was suspended in lysis buffer-1 (Tris EDTA and SDS; pH 4.0) and centrifuged at 10,000 rpm for 10 min. After centrifugation, 500 µL of chloroform: isoamyl alcohol (24:1) was added, mixed, and centrifuged at 10,000 rpm for 10 min. To the collected supernatant 1/10th volume of sodium acetate and 2.5 volumes of chilled 100% absolute ethanol were added, incubated at -20 °C for overnight, Next day, samples were centrifuged for 10 min at 10,000 rpm, and pellet was washed with 70% ethanol. After centrifugation, pellet was dried for 3 h at room temperature (25±2 °C) and DNA was suspended in distilled water (20 µl). For the identification of bacterial diversity, 16S rRNA primers; universal bacterial primer (27F; 5′-AGAGTTTGATCCTGGCTCAG-3′) was taken to amplify 1500 bps sequence using following PCR conditions (initial denaturation 95 °C for 10 min; cyclic denaturation at 95 °C for 30 sec; annealing at 50°C for 1 min; cyclic extension 72°C for l min 30 sec; and final extension 72°C for 7 min; 30 cycles) (de Lillo *et al*., 2006). After PCR analysis all PCR products were dispatch to Macrogen, Korea for sequence analysis. Obtained nucleotide sequences were further preceded for homology through BLAST at National Center for Biotechnology Information (NCBI) platform. The evolutionary history was concluded through Tamura-Nei model and neighbour-joining method (Tamura *et al*., 2007). This analysis contained 32 nucleotide sequences. There were 1573 positions in the final dataset. Evolutionary studies were accompanied in MEGA X (Kumar *et al*., 2018). After homology prediction and phylogenetic analysis, amplified sequences were submitted to Genbank, NCBI for obtaining accession numbers.

Antibiogram analysis

The emergence of multi-drug resistant among microbial biofertilizers is serious concern in developing countries now a days due to anthropogenic activities. Antibiotic resistant biofertilizers carry antibiotic resistant genes (ARGs) which aggravate negative impact not only on environment as well as on wildlife and public health (Mahdi *et al*., 2022). So, in the current study, various antibiotics like kanamycin (30µg), norfloxacin (10µg), erythromycin (15µg), amoxicillin (30µg), ampicillin (25µg), trimethoprim (5µg), penicillin (10µg), tobramycin (10µg), sulfonamide (300µg) and aztreonam (30µg) were used to display the sensitivity/resistivity of vermibacteria using agar disc diffusion method (Bauer *et al*., 1959; Brown and Kothari, 1975). Nutrient agar (Oxoid: CMOO3) and nutrient broth media (Oxoid: CM1) were used for bacterial growth. The bacteria were added to a nutrient broth medium for the growth and incubated for 24 h on a rotary shaker at 37°C. The incubated culture was mixed in a freshly prepared nutrient agar medium (NAM) at 45°C. The mixture was poured into sterilized Petri dishes, solidified in laminar flow at room temperature. After solidification, antibiotic discs were placed on the surface and then incubated for 24 h at 37ºC. According to Seeley *et al*. (2001), the growth of bacteria was determined in 24-48 h, and the diameter of the inhibition zone in mm was also measured with the help of ruler (Hammer *et al*., 1999).

Statistical analysis

Each experiment was repeated in triplicates and Mean ± Standard Deviation from absolute data was calculated: (http://easycalculation.com/statistics/standard-deviation.php).

RESULTS

Morphological features

The isolated bacterial isolates were characterized by morphological features, microscopic studies, and biochemical characterizations (Table I). Results revealed that different colonies were appeared on the various agar culture media plates i.e., MacConkey agar and nutrient agar medium. The colonies of vermicompost associated bacteria were found with different colors, size, elevations, texture, shape, and edge, respectively. The colonies of VAB showed yellow, opaque, white, cream, and cherry in color; the surface and shape of colonies were recorded as spherical, rough, oval, smooth, circular, and oval. On the

other hand, colony elevations (convex, flat, umbonate), margins (entire, irregular, filiform), and wet texture were recorded. Gram staining indicated that VAB-1, VAB-3, VAB-4, VAB-10, and VAB-12 were observed as Gram-negative rod bacteria while VAB-2, VAB-5, VAB-6, VAB-7, VAB-8, VAB-9, VAB-11, and VAB-14 are rod shaped Gram-positive bacteria.

Biochemical characterization

Results revealed that all vermicompost associated bacteria were shown catalase and oxidase positive results. In case of proteolytic assay, VAB-1, VAB-4, VAB-5, VAB-6, VAB-8, VAB-9, VAB-10, VAB-11, VAB-12, and VAB-14 were shown positive hydrolysis of casein in the range of 2.0±0.0 mm to 8.0±0.0 mm, while VAB-2, VAB-3, VAB-7 showed negative results. Similarly, positive lipolytic activity was shown by all tested VAB isolates. All VAB isolates showed amylolytic activity except VAB-3. VAB-1, VAB3, VAB-4, and VAB-9 showed urease and citrate positive results (Table I). All VAB showed the mannitol fermentation when streaked on the mannitol salt agar medium except VAB-1, VAB-2, and VAB-3 (Table I). Hemolytic test results revealed that all isolated VABs are non-pathogenic in nature.

Plant growth promoting traits

Results revealed that all VAB isolates were involved in the production of siderophore and Indole acetic acid (except VAB-6), and act as phosphate solubilizes (Table II). Ammonia production test revealed that VAB-1, VAB-6, VAB-7, VAB-8, VAB-9, VAB-10, VAB-12, and VAB-14 were positive for this test. Yellow color was observed in the medium, denoting the production of ammonia by these isolates. All VAB involved in the HCN production except VAB-6 (Table II). In case of KOH test, VAB-3, VAB-4, and VAB-6 showed negative results.

Molecular identification

The 16S rDNA genes from isolated bacteria were amplified using 27F bacterial universal primer and the size of amplified genes was recorded in the range of 1100 bps to 1769 bps. The partial nucleotide sequences were proceeded for further BLAST analysis at National Center for Biotechnology Information (NCBI) and results reveal that no significant similarities of VAB-8, VAB-9, and VAB-11 were found (Table III). On the other hand, VAB-1 indicated 91% similarity with *Achromobacter insolitus* (AB642187.1), *Achromobacter denitrificans* (KY475779.1), *Achromobacter anxifer* (MN197585.1), *Achromobacter ruhlandii* (KY087962.1), *Achromobacter agilis* (MK791141.1), and *Achromobacter xylosoxidans* (MK587674.1); VAB-2 showed 99.55% homology with *Bacillus cereus* (JX971533.1) and 99.46% with

Table I. Morphological and biochemical characterization of vermicompost associated bacteria.

Characteristics → Bacterial strains ID↓	GS	Sha	Color	Colony sha/sur	Ele	Mar	Tex	MSA	Mot	Cata	Oxid	Amy	Pro	Lip	Ure	Cit
Achromobacter ruhlandii (VAB-1)	-	Rod	Yel	Sph/Rou	Con	Ent	Wet	-	+	+	+	+	+	+	+	+
Bacillus wiedmannii (VAB-2)	+	Rod	Opa/W	Oval/Sm	Flat	Irg	Wet	-	+	+	+	+	-	+	-	+
Pseudomonas sp. (VAB-3)	-	Rod	Yel	Sph/Rou	Flat	Ser	Dry	-	+	+	+	-	-	+	+	+
Achromobacter xylosoxidans (VAB-4)	-	Rod	Opa	Oval/Sm	Con	Ent	Wet	+	+	+	+	+	+	+	+	+
Oceanobacillus oncorhynchi (VAB-5)	+	Rod	Yel	Cir/Sm	Con	Ent	Wet	+	+	+	+	+	+	+	-	-
Bacillus mycoides (VAB-6)	+	Rod	W	Cir/Sm	Flat	Irg	Wet	+	-	+	+	+	+	+	-	+
Serratia nematodiphila (VAB-7)	+	Rod	Cre	Cir/Sm	Con	Ent	Wet	+	+	+	+	+	-	+	-	+
Unknown (VAB-8)	+	Rod	Yel	Oval/Sm	Con	Ent	Wet	+	+	+	+	+	+	+	-	+
Unknown (VAB-9)	+	Rod	Yel	Oval/Sm	Con	Ent	Wet	+	+	+	+	+	+	+	+	+
Serratia marcescens (VAB-10)	-	Rod	Cre	Cir/Sm	Umb	Filli	Wet	+	+	+	+	+	+	+	-	+
Unknown (VAB-11)	+	Rod	Cre	Cir/Rou	Con	Ent	Wet	+	+	+	+	+	+	+	+	+
Serratia marcescens (VAB-12)	-	Rod	Cre	Cir/Sm	Umb	Filli	Wet	+	+	+	+	+	+	+	-	+
Paenibacillus dendritiformis (VAB-14)	+	Rod	Che	Oval/Rou	Con	Ent	Wet	+	+	+	+	+	+	+	-	+

GS, Gram staining, Sha, Shape of bacteria; Sur, Surface; Ele, Elevation; Mar, Margin; Tex, Texture; MSA, Mannitol salt agar; Mot, Motility, Cata, Catalase, Oxid, Oxidase; Amy, Amylase, Pro, Proteases, Lip, Lipase; Ure, Urease; Cit, Citrate; Yel, Yelow, W, White; Opa, Opaque; Cre, Cream; Sm, Smooth; Umb, Umbonate; Filli, Filliform; Con, Convex; Ent, Entire, W, White, Irg, Irregular; Cir, Circular; Rou, Rough; SPh, Spherical; +, positive; -, negative.

Table II. Plant growth promoting traits of vermicompost associated bacteria.

Agricultural traits → Bacterial strains ID↓	Sidero-phore	Phos-phate	Potassium hydroxide	Indole acetic acid	Hydrogen cyanide	Ammo-nia
Achromobacter ruhlandii (VAB-1)	+	+	+	+	+	+
Bacillus wiedmannii (VAB-2)	+	+	+	+	+	-
Pseudomonas sp. (VAB-3)	+	+	-	+	+	-
Achromobacter xylosoxidans (VAB-4)	+	+	-	+	+	-
Oceanobacillus oncorhynchi (VAB-5)	+	+	+	+	+	-
Bacillus mycoides (VAB-6)	+	+	-	-	-	+
Serratia nematodiphila (VAB-7)	+	+	+	+	+	+
Unknown (VAB-8)	+	+	+	+	+	+
Unknown (VAB-9)	+	+	+	+	+	+
Serratia marcescens (VAB-10)	+	+	+	+	+	+
Unknown (VAB-11)	+	+	+	+	+	-
Serratia marcescens (VAB-12)	+	+	+	+	+	+
Paenibacillus dendritiformis (VAB-14)	+	+	+	+	+	+

Table III. BLAST outcomes of vermicompost associated bacteria.

Microbe coding	Amplified length	Percent identity (%)	Scientific name	Sequence id	Accession length
VAB-1	1170	91%	*Achromobacter insolitus*	AB642187.1	1295
		91%	*Achromobacter denitrificans*	KY475779.1	1339
		91%	*Achromobacter anxifer*	MN197585.1	1420
		91%	*Achromobacter ruhlandii*	KY087962.1	1448
		91%	*Achromobacter agilis*	MK791141.1	1398
		91%	*Achromobacter xylosoxidans*	MK587674.1	1426
VAB-2	1171	99.55	*Bacillus cereus*	JX971533.1	1374
		99.46	*Bacillus subtilis*	MG593998.1	1188
		99.46	*Bacillus tropicus*	ON878141.1	1351
		99.46	*Bacillus thuringiensis*	ON974260.1	1423
		99.46	*Bacillus paranthracis*	ON939702.1	1419
		99.46	*Bacillus pacificus*	ON885910.1	1417
		99.46	*Bacillus wiedmannii*	ON340605.1	1370
		99.46	*Bacillus paramycoides*	ON000574.1	1448
VAB-3	1679	94	*Bacillus firmus*	LC385949.1	710
		89	*Escherichia coli*	MH793567.1	1371
		93	*Stenotrophomonas maltophilia*	MK493713.1	643
		93	*Pseudomonas* sp.	MT793102.1	1498
VAB-4	1224	96.82	*Achromobacter insolitus*	MN044760.1	1300
		96.52	*Achromobacter denitrificans*	MT275805.1	1374
		96.52	*Achromobacter ruhlandii*	KY087962.1	1448
		96.56	*Achromobacter xylosoxidans*	MK587674.1	1426
		96.09	*Achromobacter aegrifaciens*	NR_117707.1	1483

Table continues on next page.....

Microbe coding	Amplified length	Percent identity (%)	Scientific name	Sequence id	Accession length
VAB-5	1100	78.95	*Bacillus pumilus*	KM222185.1	1448
		78	*Bacillus subtilis*	OM760707.1	1454
		83	*Bacillus cereus*	FJ404785.1	539
		77	*Oceanobacillus oncorhynchi*	JX280491.1	1485
		75	*Pediococcus pentosaceus*	ON545700.1	1102
VAB-6	1100	81	*Bacillus anthracis*	KP813652.1	1221
		81	*Bacillus toyonensis*	LN995802.1	1111
		81	*Bacillus thuringiensis*	HM032789.1	948
		81	*Bacillus cereus*	MF726967.1	1267
		80	*Bacillus proteolyticus*	MK418822.1	1028
		80	*Bacillus albus*	MN658863.1	1235
		81	*Bacillus manliponensis*	MW519873.1	1083
		81	*Bacillus pseudomycoides*	MT573519.1	1040
		82	*Bacillus mycoides*	KU687331.1	1257
VAB-7	1100	87.03	*Serratia marcescens*	MF462933.1	1479
		87	*Staphylococcus aureus*	MH603394.1	1161
		87	*Serratia surfactantfaciens*	NR_169468.1	1495
		87	*Enterobacter cloacae*	KJ850207.1	1434
		87	*Raoultella planticola*	MZ203710.1	1363
		87	*Serratia nematodiphila*	KM099143.1	1392
		87	*Klebsiella* sp.	MZ203699.1	1363
VAB-8	1100	No significant similarity found			
VAB-9	1100	No significant similarity found			
VAB-10	1100	83.16	*Serratia marcescens*	MT386168.1	1385
		83.16	*Pseudomonas* sp. *strain jx-18*	KY780232.1	1454
		83.16	*Serratia nematodiphila*	ON651725.1	1261
VAB-11		No significant similarity found			
VAB-12	1591	94	*Serratia marcescens*	KM099142.1	1411
		92	*Serratia nematodiphila*	MN691576.1	1251
VAB-14	1100	89	*Paenibacillus dendritiformis*	MG592704.1	1414
		89	*Paenibacillus popilliae*	KC107790.1	1380
		89	*Paenibacillus thiaminolyticus*	EU330645.1	1474

Bacillus subtilis (MG593998.1), *Bacillus tropicus* (ON878141.1), *Bacillus thuringiensis* (ON974260.1), *Bacillus paranthracis* (ON939702.1), *Bacillus pacificus* (ON885910.1), *Bacillus wiedmannii* (ON340605.1), *Bacillus paramycoides* (ON000574.1); VAB-3 showed 94% resemblance with *Bacillus firmus* (LC385949.1), 89% with *Escherichia coli* (MH793567.1), 93% *Stenotrophomonas maltophilia* (MK493713.1), 93% with *Pseudomonas* sp. (MT793102.1); VAB-4 showed 96.82% similarity with *Achromobacter insolitus* (MN044760.1), 96.52% with *Achromobacter denitrificans* (MT275805.1), 96.52% with *Achromobacter ruhlandii* (KY087962.1), 96.56% with *Achromobacter xylosoxidans* (MK587674.1), 96.09% with *Achromobacter aegrifaciens* (NR_117707.1); VAB-5 indicated 78.95% similarity with *Bacillus pumilus* (KM222185.1), 78% with *Bacillus subtilis* (OM760707.1), 83% with *Bacillus cereus* (FJ404785.1), 77% with *Oceanobacillus oncorhynchi* (JX280491.1), 75% with *Pediococcus pentosaceus* (ON545700.1); VAB-6 showed 81% homology with *Bacillus anthracis* (KP813652.1), *Bacillus toyonensis* (LN995802.1), *Bacillus thuringiensis* (HM032789.1), *Bacillus cereus* (MF726967.1), *Bacillus*

manliponensis (MW519873.1), *Bacillus pseudomycoides* (MT573519.1), 80% with *Bacillus proteolyticus* (MK418822.1), *Bacillus albus* (MN658863.1), 82% with *Bacillus mycoides* (KU687331.1); VAB-7 displayed 87.03% resemblance with *Serratia marcescens* (MF462933.1), 87% with *Staphylococcus aureus* (MH603394.1), *Serratia surfactantfaciens* (NR_169468.1), *Enterobacter cloacae* (KJ850207.1), *Raoultella planticola* (MZ203710.1), *Serratia nematodiphila* (KM099143.1), *Klebsiella* sp. (MZ203699.1); VAB-10 indicated 83.16% similarity with *Serratia marcescens* (MT386168.1), *Pseudomonas* sp. strain jx-18 (KY780232.1), *Serratia nematodiphila* (ON651725.1); VAB-12 showed 94% resemblance with *Serratia marcescens* (KM099142.1) and 92% with *Serratia nematodiphila* (MN691576.1); and VAB-14 indicated 89% homology with *Paenibacillus dendritiformis* (MG592704.1), *Paenibacillus popilliae* (KC107790.1), and *Paenibacillus thiaminolyticus* (EU330645.1). The results of phylogenetic tree using the NJ method were recorded as VAB-1 indicated relationship with *Achromobacter ruhlandii* (81%), VAB-2 with *Bacillus wiedmannii* (81%), VAB-3 with *Pseudomonas* sp. (82%); VAB-4 with *Achromobacter xylosoxidans* (96%), VAB-5 with *Oceanobacillus oncorhynchi* (74%), VAB-6 with *Bacillus mycoides* (87%), VAB-7 with *Serratia nematodiphila* (74%), VAB-10 with *Serratia marcescens* (95%), VAB-12 with *Serratia marcescens* (79%), and VAB-14 with *Paenibacillus dendritiformis* (75%).

Antibiogram analysis

Erythromycin and norfloxacin showed the maximum inhibition of tested vermicompost associated bacteria (Table IV). Erythromycin showed the maximum inhibition of *Achromobacter ruhlandii* (18.0±0.0 mm), *Pseudomonas* sp. (14.0±0.0 mm), *Oceanobacillus oncorhynchi* (14.0±0.0 mm), *Bacillus mycoides* (12.0±0.0 mm), unknown (18.0±0.0 mm), unknown (18.0±0.0 mm), *Serratia marcescens* (20.0±0.0 mm), unknown (16.0±0.0 mm), *Serratia marcescens* (20.0±0.0 mm). Similarly, norfloxacin indicated the maximum inhibition of *Achromobacter ruhlandii* (14.0±0.0 mm), *Achromobacter xylosoxidans* (13.0±0.0 mm), *Bacillus mycoides* (11.0±0.0), Unknown (15.0±0.0 mm), unknown (14.0±0.0 mm), *Serratia marcescens* (13.0±0.0 mm), unknown (17.0±0.0 mm), *Serratia marcescens* (11.0±0.0 mm) *Paenibacillus dendritiformis* (17.0±0.0 mm). Trimethoprim, kanamycin, sulfonamide showed the maximum inhibition of *Oceanobacillus oncorhynchi* and VAB-11 with 17.0±0.0 mm, 15.0±0.0 mm, 11.0±0.0 mm, 19.0±0.0 mm, 14.0±0.0 mm, 12.0±0.0 mm. On the other hand, all tested VAB isolates displayed resistant towards trimethoprim, aztreonam, ampicillin, and amoxocillin (Table IV). Tobramycin and penicillin showed lowest and moderate inhibition of tested vermicompost associated bacterial isolates.

Table IV. Antibiogram analysis of vermicompost associated bacteria.

Used Antibiotics → **Bacterial strains↓**	**Zone of inhibition (mm) M±SD**									
	TMP (5µg)	**ATM (30µg)**	**K (30µg)**	**E (15µg)**	**S (300µg)**	**TOB (10µg)**	**P (10µg)**	**NOR (10µg)**	**AMC (30µg)**	**AMP (25µg)**
Achromobacter ruhlandii (VAB-1)	0.0±0.0	0.0±0.0	9.0±0.0	18.0±0.0	10.0±0.0	5.0±0.0	5.0±0.0	14.0±0.0	0.0±0.0	0.0±0.0
Bacillus wiedmannii (VAB-2)	0.0±0.0	0.0±0.0	9.0±0.0	6.0±0.0	14.0±0.0	7.0±0.0	0.0±0.0	9.0±0.0	0.0±0.0	0.0±0.0
Pseudomonas sp. (VAB-3)	0.0±0.0	0.0±0.0	7.0±0.0	14.0±0.0	12.0±0.0	6.0±0.0	0.0±0.0	10.0±0.0	0.0±0.0	0.0±0.0
Achromobacter xylosoxidans (VAB-4)	0.0±0.0	0.0±0.0	5.0±0.0	0.0±0.0	0.0±0.0	5.0±0.0	0.0±0.0	13.0±0.0	0.0±0.0	0.0±0.0
Oceanobacillus oncorhynchi (VAB-5)	17.0±0.0	0.0±0.0	15.0±0.0	14.0±0.0	11.0±0.0	6.0±0.0	19.0±0.0	7.0±0.0	0.0±0.0	0.0±0.0
Bacillus mycoides (VAB-6)	0.0±0.0	0.0±0.0	6.0±0.0	12.0±0.0	12.0±0.0	6.0±0.0	7.0±0.0	11.0±0.0	0.0±0.0	0.0±0.0
Serratia nematodiphila (VAB-7)	0.0±0.0	0.0±0.0	5.0±0.0	3.0±0.0	4.0±0.0	5.0±0.0	10.0±0.0	10.0±0.0	0.0±0.0	0.0±0.0
Unknown (VAB-8)	0.0±0.0	0.0±0.0	6.0±0.0	18.0±0.0	6.0±0.0	6.0±0.0	2.0±0.0	15.0±0.0	0.0±0.0	0.0±0.0
Unknown (VAB-9)	0.0±0.0	0.0±0.0	5.0±0.0	18.0±0.0	7.0±0.0	6.0±0.0	0.0±0.0	14.0±0.0	0.0±0.0	0.0±0.0
Serratia marcescens (VAB-10)	0.0±0.0	0.0±0.0	3.0±0.0	20.0±0.0	6.0±0.0	5.0±0.0	0.0±0.0	13.0±0.0	0.0±0.0	0.0±0.0
Unknown (VAB-11)	19.0±0.0	0.0±0.0	14.0±0.0	16.0±0.0	12.0±0.0	15.0±0.0	0.0±0.0	17.0±0.0	0.0±0.0	0.0±0.0
Serratia marcescens (VAB-12)	0.0±0.0	0.0±0.0	5.0±0.0	20.0±0.0	4.0±0.0	4.0±0.0	10.0±0.0	11.0±0.0	0.0±0.0	4.0±0.0
Paenibacillus dendritiformis (VAB-14)	0.0±0.0	0.0±0.0	16.0±0.0	8.0±0.0	8.0±0.0	5.0±0.0	12.0±0.0	17.0±0.0	5.0±0.0	5.0±0.0

DISCUSSION

Vermicompost associated bacteria

Based on the morphological, microscopic, biochemical tests, and molecular characterization, 10 VAB strains were identified *viz. Achromobacter ruhlandii*, *Bacillus wiedmannii*, *Pseudomonas* sp., *Achromobacter xylosoxidans*, *Oceanobacillus oncorhynchi*, *Bacillus mycoides*, *Serratia nematodiphila*, *Serratia marcescens,* and *Paenibacillus dendritiformis*. The current results agreed with previous literature (Upadhyay *et al*., 2015; Selvi and Koilraj, 2015; Begum and Bora, 2018). Khyade (2018) and Vaz-Moreira *et al*. (2008) also demonstrated the presence of *B. cereus, B. pumilus, B. macrolides, B. licheniformis, Bacillus benzoevorans, B. megaterium,* and *B. subtilis* from vermicompost. Several bacteria i.e. *Pseudomonas, Klebsiella, Bacillus, Serratia, Azospirillum, Acetobacter, Burkholderia*, and *Azotobacter*, have been recorded as plant growth-promoting bacteria (PGPB) while *Pseudomonas* and *Bacillus* spp. have been identified as the predominant communities. Similar results were found by Kang *et al*. (2014), and our findings agreed with them. Andleeb *et al*. (2022) isolated and identified eleven vermibacteria from the gut of *E. fetida* such as *B. megaterium, Bacillus mycoides, Staphylococcus hominis, B. aryabhattai, B. subtilis, B. licheniformis, B. spizizenii, B. mojavensis, B. cereus, B. toyonensis, B. thuringiensis*, *B. anthracis,* and *B. paranthracis.* which enhanced the quality of vermicompost during vermicomposting?

Vermicompost associated bacteria possessed agricultural traits

Vermibacteria associated with the gut of *E. fetida* possessed agricultural traits and their impact was done on ornamental plants. These plant growth promoting vermibacteria (PGPVB) not only used for the develop of plant growth but also enhance the plant nutrition value either directly or indirectly mechanisms (Andleeb *et al*. 2022). The direct assistance of plant growth is carried out by various ways such as supplementation of essential nutrients such as phosphate, nitrogen, potassium, zinc, and iron (Divjot *et al*., 2020; Fasciglione *et al*., 2015). Similarly, PGPB also enhanced the plant growth by the production of phytohormones (Al-Kahtani *et al*., 2020). On the other side, the indirect plant growth promotion occurs through the prevention of deleterious effects of pathogens and pest on plants by releasing compounds or defense enzymes/ proteins by PGPBs (Simon *et al*., 2019; Frampton *et al*., 2012). Several reports have also been known for the imperative role of PGPRs in mitigating salt stress in different crop plants tomato, groundnut, wheat, rice and red pepper (Upadhyay and Singh, 2015; Bal *et al*., 2013; Shukla *et al*., 2012). The current results showed that vermicompost associated bacterial strains i.e., *Achromobacter ruhlandii*, *Bacillus wiedmannii*, *Pseudomonas* sp., *Achromobacter xylosoxidans*, *Oceanobacillus oncorhynchi*, *Bacillus mycoides*, *Serratia nematodiphila*, *Serratia marcescens,* and *Paenibacillus dendritiformis* have ability to produce siderophores, hydrolytic enzymes, hydrogen cyanide, ammonia, and IAA, and solubilize phosphates and our findings agreed with the outcomes of Mahdi *et al*. (2020) and Ahemad and Kibret (2014). *Achromobacter* spp. are endophytic bacteria, showed plant growth promoting properties like production of IAA, HCN, and ammonia, and have phosphate solubilizing activity. *Oceanobacillus oncorhynchi* is gram-positive rod shaped endophytic bacteria, and also possessed agricultural traits. Our outcomes agreed with previous reports indicated that *Oceanobacillus oncorhynchi* and *Achromobacter spp* could be used as PGPB (Mapelli *et al*., 2013; Orhan, 2016; Jha and Kumar, 2009).

Key role of Vermicompost associated bacteria as PGPB

The current study reveals that vermicompost associated bacteria possessed osphate solubilizing capability and phosphorous solubilizing microbes play an important role directly and indirectly in biological, physical, and chemical soil properties, and agreed with previous studies (Guo *et al*., 2015; Alori *et al*., 2017). The presence of phosphate solubilizing bacterium in soils as well as vermicompost may be considered a positive indicator of utilizing the microbes as biofertilizers for crop production and beneficial for sustainable agriculture. In addition, phosphorous solubilization, VAB involved in the IAA and siderophore production which promotes the seed germination, enhance root development, biosynthesis of various metabolites, initiate florescence, enhances root surface area and root length, resistance to biotic and abiotic stresses, and nodule formation. Bacterial isolates also able to produce ammonia which indicates that VAB supply nitrogen to their plants and not only capable to promote shoot and root elongation as well as biomass of the plant, and our findings are consistent with the outcomes of Etesami and Beattie (2018), Mahanty *et al*. (2017), and Dutta *et al*. (2015). The nitrogen fixation ability of associative bacteria provides the essential nitrogen content during the growth phase of the plant (Jha and Kumar, 2009). It was observed that *Bacillus* species such as *B. licheniformis*, *B. cereus,* and *B. subtilis* involved in the enhancement of root colonization, phytohormone production, enhanced the plant growth and possessed biocontrol properties due to presence of agricultural traits. Our findings agreed with previous (Radhakrishnan and Lee, 2016; Allard-Massicotte *et al*., 2016; Rijavec and

Lapanje, 2016; Islam *et al*., 2014; Beauregard *et al*., 2013).

VABs are source of spread of antibiotic resistant genes

The major concern on the development of multi-drug resistant microbes is increasing day by day due to extensive use of antibiotics in livestock. These antibiotics alter the gut microbiota of the animals that started to attain antibiotic resistance and raises the transfer of antibiotic resistant genes to rhizosphere bacteria through horizontal gene transfer (Tariq *et al*., 2022). These bacteria might be involved in the spread of resistome to human pathogens or can infect humans through contaminated vegetables and fruits (Checcucci *et al*., 2020; Zeng *et al*., 2018). According to Gonzalez *et al*. (2017), some PGPB involved in the human infections on exposure of contaminated soil, water and farm products. So, in the current study, screening of antibiotic susceptibility tests was necessary to know the status of VABs that could be used as microbial biofertilizers. Results revealed that all vermibacterial isolates are not multidrug resistant bacteria and their presence in the fields reduced the possibility of transfer of resistome in foodchain.

Current study reveals that vermicompost associated bacteria i.e., *Achromobacter* spp., *Bacillus* spp., *Pseudomonas* sp., *Oceanobacillus oncorhynchi, Serratia* spp., and *Paenibacillus dendritiformis* could be used as microbial biofertilizers to enhance crop production, involved in vermicomposting, improves the vermicompost quality and soil health, and used as a biocontrol agent due to presence of agricultural traits.

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Ethical statement and approval

All experiments conducted during the research were carefully designed to prevent distress, suffering, and unnecessary pain to the experimental animals. All procedures were carried out in accordance with international regulations, specifically following Article 9 of the Dutch Law on animal experimentation (Wet op de dierproeven). The current study was approved by Institutional Review Committee of Office of Research, Innovation, and Commercialization (ORIC), The University of Azad Jammu and Kashmir, Muzaffarabad vide No. 246/ORIC/2022; Dated: 3-10-2022.

Availability of data and materials

All the data generated during this study are included in this article.

Supplementary material

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/20230525060540

Statement of conflict of interest

The authors have declared no conflict of interest.

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Supplementary Material

Molecular Depiction of Vermicompost Associated Bacteria Possessed Agricultural Traits

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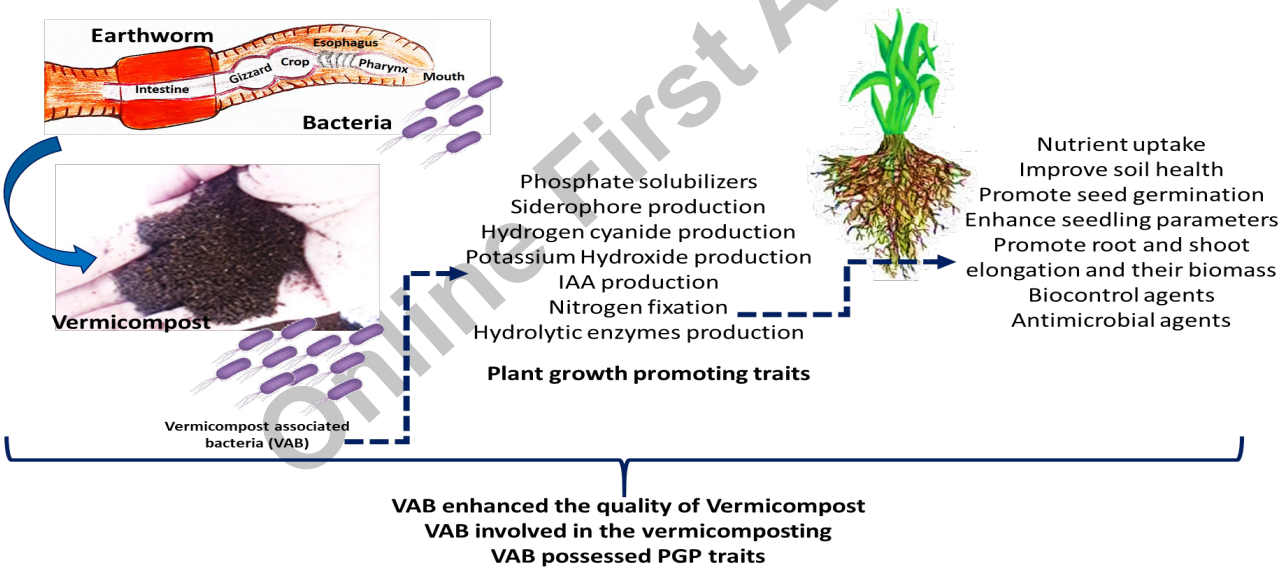

Supplementary Fig. 1. Production and importance of vermicompost associated bacteria as plant growth promoting bacteria.

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