Innovations

Isolation of Heavy Metal Tolerating Bacteria and Its Effect on Two Different Wheat Varieties

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Abstract: *Heavy metal contamination is one the various reasons for cancer across the globe. Heavy metal load in soil is increasing day by day via various ways including application of chemical fertilizers in crop plants. In current research, attempts were made to isolate the heavy metal tolerating plant growth promoting bacteria. A total of 22 bacterial isolates were obtained from the heavy metal polluted industrial soils from Chandigarh region of India. Isolates were characterized biochemically and were subjected to plant growth promoting tests. Out of total, 59.1% and 50% isolates found positive zinc and phosphate solubilisation. A total of 63.6% isolates found siderophore producers while HCN production was observed in 45.4% isolates. Indole acetic acid (IAA) production was recorded in 68.1% isolates. A total of 4 isolates exhibited 4 or more than 4 plant growth promoting traits and were identified at molecular level using amplification and sequencing of 16S rDNA sequences. The DNA sequences are submitted on NCBI GenBank and accession numbers are obtained. The selected isolates were inoculated with wheat varieties PBW826 and 3086 grown in the sterile pots containing normal as well as heavy metal polluted soil. It was observed that inoculated plant showed significant increase in growth, growth related parameters and yield as compared uninoculated control plants. These isolates could become very crucial for the growth and yield of wheat crop for sustainable agriculture, especially in heavy metal polluted areas. Though, field trials are required to uncover the potential of isolates for plant growth and yield in open uncontrolled environment.*

Discipline: *Agricultural Microbiology*

Keywords*: Heavy metal, PGPB, wheat, soil pollution, plant growth*

Running title: Isolation of microbes from heavy metal polluted area and their effect on plant growth promotion

Introduction

Heavy metals are metallic elements besides alkali and alkaline earth that could be harmful to people or the environment. Heavy metals are highly dangerous compounds in nature because of their low density. Since heavy metals are non-biodegradable, they pose a serious hazard to both human well-being and the environment (Gumpu *et al.* 2015). They keep on accumulating in the biosphere, contaminate living organisms via food chain, and have an effect on people's health because they are naturally non-biodegradable (Gumpu *et al.* 2015). Heavy metals like cadmium (Cd), arsenic (As), chromium (Cr), lead

(Pb), and mercury (Hg), are considered to be hazardous chemical compounds even at low quantities. The chief sources of these heavy metals include chemical constituents used in cosmetic industries, various radiations, industrial wastes, etc. The impacts of heavy metals on cellular functions when they enter the human body from diverse sources are what cause heavy metal poisoning. While certain metals are excreted through the liver, kidney, or spleen, others accumulate in some excretory organs and harm the organs. Heavy metal is the major cause of increasing cases of cancer in Punjab region. Heavy metal plays a very important part in food contamination that is why it is one of the main reasons why there are still concerns about food security. The main food contaminants include; heavy metal contamination, pesticides, and toxins (Wang *et al.* 2019).

Wheat (*Triticum aestivum* L.) has a substantial nutritional significance for human existence because wheat is a staple food and accounts for 70% of the food produced worldwide (Kianpour and Sobhanardakani 2018). About 14th of the grain produced annually is wheat. It is a major source of food in many countries including India thus provides more than half of the calories needed by its growing population (Khan *et al.* 2015) Like various plants, wheat growth is also sensitive to heavy metals contamination in the soil (Ghani*et al.* 2015; Stanišić *et al*. 2016). When heavy metals enter plants, the stress they endure results in a variety of impacts including disturbances to germination, metabolism, development and growth, (Paunov *et al.* 2018) as well as reduced wheat yields (Sindhu *et al.* 2015; Singh *et al.* 2014).

Plant growth-promoting bacteria (PGPB) constitute important participants in agriculture. It promotes the health of crops by fixing atmospheric nitrogen, increasing phosphate solubilization, decreasing heavy metal concentration, producing phytohormones like auxin, gibberellins, cytokinins, etc., mineralizingorganic matter present in the soil, decomposition of crop residue, inhibiting phytopathogens, and carrying out other functions (Etesami and Maheshwari 2018; He *et al.* 2019). Plant development is slowed down by metal pollutants in the rhizosphere that inhibit plants from obtaining nutrients (Lal *et al.* 2018). It leads to low level of development and ultimately decrease in the yield. This could be overcome by inoculating a PGPB containing metal resistant trait. Plants can be successfully protected from abiotic stress when PGPB is activated. Improved growth conditions and yield were achieved while managing heavy metal stress with inoculationof PGPB (Guo *et al.* 2020).

The aim of the present study is the isolation and identification of heavy metal tolerating PGPB and their impact on the growth and development of the wheat crop for sustainable agriculture in heavy metal polluted soil.

Material and methods

Sample collection

A total of twenty-two PGPB isolates were characterized in this study. For the purpose of this project work, four samples of soil were collected from different industriesof Chandigarh *i.e.,* Ravindra industries, Aggarwal metal company, Aarsh welding works, Gurdeep industries having lat-long 30.69^0 N, 76.78^0 E. Samples were collected from upper layer (2-4cm) where most of heavy metal is concentrated.

Isolation of microbes

Isolation of the microbes from soil samples was carried out using serial dilution method (Cullen and MacIntyre2016). The isolated microbes were spread on the plate containing Nutrient agar media (NAM) and were subsequently incubated at 37± 2°C for 24 hours. In order to obtain the pure cultures, sub-culturing was done repeatedly using single colonies. All the isolates obtained were preserved at 4°C in media containing equal volumes of nutrient broth and 30% glycerol.

Biochemical Identification

Identification of different bacterial isolates was done based onthe morphology (Jida and Assefa 2012)and biochemical tests (Table7) such as indole production test, Methyl red reaction test and Voges-Proskauer test (MR-VP), Gram's staining, Citrate utilization test, Catalase test, Protease test, Oxidase test, Urease test (Sharma *et al.* 2017).

In-vitro PGP characterization

After performing different biochemical tests these isolates were evaluated for production of biocontrol and plant growth promoting traits such as siderophores, phosphate and zinc solubilization (Schwyn and Neilands 1987), hydrocyanic acid (HCN) (Bakker and Schippers 1987), indole acetic acid (IAA) (Bano and Musarrat 2003), Chitinase tests (Table 8). The most promising bacterial isolates were sent for molecular identification.

Molecular characterization

Molecular identification was carried out by amplification and sequencing of 16s rDNA sequence. The universal primer pairs 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-GTTACCTTGTTACGACTT-3') were used for gene amplification of 16S rRNA. DNA sequencing was carried out at National Bureau of Agriculturally important microbes (ICAR-NBAIM, Mau, India).In order to determine the similarity percentile,the sequences obtained were compared by $BLAST_N$ search against NCBI database.

Phylogenetic study of bacterial isolates

By using ClustalW program (MEGA version 11) the alignment of gene sequence was done (Fitzgerald *et al.* 2020). The test of phylogeny was carried out with the help of bootstrap method having 1000 no. of bootstrap replications of the sequences.The evolutionary history was inferred using the Neighbor-Joining method(Fig1). The obtained gene sequences of 16S rRNA were deposited in the NCBI database in order to attain their accession numbers Table1.

Inoculation of isolated bacteria

The fertile loamy soil was collected from the field near Chandigarh university, Punjab and the heavy metal containing soil was collected from Industrial area phase II Chandigarh. The collected soil samples were taken to laboratory and were sterilized by autoclaving, typically at 121 °C (250 °F) for 30 minutes. Soil was then fractionated and about 800gm of fertile normal loamy soil and 200 g of heavy metal containing was transferred in medium sized poly bags for sowing. Sowing was done right after the soil preparation; seeds of wheat were sown for cultivation in the month of January, 2023. The pot experiment was conducted in the green house of Chandigarh University (Punjab) during Rabi season. The wheat varieties used for the experiment were PBW826 and 3086 obtained from seed distributers from Kharar, Punjab.

After sowing seeds in different pots separately, pots were inoculated with different types of Bacterial strains individually like *Pseudomonas putida, Pseudomonas protegens, Bacillus rhizoplanae.* About 5ml of the broth containing bacterial strains were used per seed of wheat in the pot. After the incubation of potential microbes, the plant growth and development were observed and weight and height of plantlets was noted (Table 10-11).

Results

Soil characteristics

From the physicochemical analysis of soil samples (Table 2),it is observed that soil sample had slightly alkaline pH ranges from (7.32± 1.25 to 8.21 ± 1.33), electrical conductivity (from 0.26 to 0.32 ds/m). The color of soil samples varied from black to dark brown. The characterization of soil samples was done on the basis of concentration of organic matter (from 0.03 ± 0.01 to 0.04 ± 0.02 g/kg of soil), organic carbon (from 0.42 ± 0.12 to 0.62 ± 0.20 g/kg of soil), and total nitrogen (from 0.77 ± 0.35 to 1.07 ± 0.46 g/kg of soil). Heavy metal detection test shows the high amount of arsenic and chromium in soil samples (Table 3).

Analysis of microbial population on plate

In order to obtain the heavy metal tolerating bacteria, the serial dilution of the soil samples collected from the industries was done. To enhance bacterial isolation, sterile pipette was used to remove 1ml of each dilution and introduced into the surface of the NAM. Plates were then incubated at 37^0C for 24 hours (or until they reached full growth). Visual and microscopic observation was used to count the bacterial colonies. The details of the colonies obtained are given in Table 4 and 5.

Colony morphology

The formed colonies had a regular or irregular shape, whereas, the color of the colonies obtained varied from milky translucent to creamy, opaque, green and orange. The isolates obtainedhad small to large colonies that under optimal growth conditionsproduce mucus (Table 6) The most of the bacterial isolatesexhibited motility, have rod like shape and weregram-negative and (Table 7).

PGP properties of the isolates

Various plant growth promoting tests were performed to know plant growth promoting traits (Table 8). Out of all twenty-two isolates, four isolates gave positive test for two PGP traits, thirteen isolates showed three positive PGP tests, one isolates gave positive test for four PGP tests, while four isolates gave positive test of all six PGP traits. It was deciphered that the four strains N1D9, N2D4, N2D6, N3D8, showed positive test for1AA, Phosphate solubilization, HCN, Zinc oxide, Chitinase, Siderophore test.

From the results obtained it is observed thatfifteen(68.1%)isolatesshow positive IAA test, eleven (50%) isolates gave positive phosphate solubilization test, ten (45.4%) isolates are observed to show positive HCN test, thirteen (59.1%) isolates gave positive zinc solubilization test, twelve (54.5%) isolates show positive chitinase test while fourteen (63.6%) isolates gave positive siderophore test.

Effect of microbial inoculation on plant growth and development

Microbial isolates showing multiple plant growth promoting traits were selected as bio-inoculants to assess their efficiency on plant growth promotion. Plants growth traits such as germination, plant height as well as dry and wet weights of root and shoot were observed. It was noticed that the plants inoculated with potential microbial strains exhibited rapid germination as well as speedy growth and development as compared to control.

In variety PBW-826, the plant inoculated with isolate N1D9, exhibited significant germination rate whereas the plant containing heavy metal soil without any inoculated bacteria showed slow germination rate.

Subsequently in this variety (PBW-826), maximum plant height was seen in plants treated with N2D6 (86.8 ± 1.9cm) (Table 9) followed by plants treated with N3D8 (85.2 ± 1.7 cm), N1D9 (84.3 ± 1.5cm), N2D4 (83.7 ± 2.1 cm), normal control (70.7 ± 2.5 cm). The minimum plant height was measured in heavy metal control (62.3 ± 1.9 cm).

In variety 3086, the plant inoculated with isolate N2D6, exhibited significant germination rate whereas the plant containing heavy metal soil without any inoculated bacteria showed slow germination rate.

In this variety, maximum plant length was seen in plants treated withN3D8 (82.2 ± 1.9cm)Table10), followed by plants treated, N2D4 (81.4 ± 2.7 cm), N1D9 (79.7 ± 2.1 cm), N2D6 (75.3 ± 0.9 cm), normal control (68.7 ± 2.4 cm). The minimum plant height was measured in heavy metal control (60.3 ± 1.8 cm).

Phylogenetic characterization

The phylogenetic characterization was done by using Blast analysis of 16s rRNA gene sequences of fourisolates that evidently revealed that three isolates fell under the genus *Pseudomonas* with 99.02-99.28%sequence similarity while one isolates fell under genus *Bacillus* with 99.44% sequence similarity (Table1). From the phylogenetic reconstruction of 16s rRNA gene sequences,it is observed that these isolates were grouped in sub group along with their reference strains of *Pseudomonas, Bacillus.* (Fig 1)

Discussion

In India about two-third of food grains is produced in Punjab, due to large fertility of the soil The south-western region of Punjab is rich in calcareous soil that consists of desert soil and alluvial soil having pH ranges from 7.8-8.5. The Central-Punjab consists of clayey and sandy loamy soil with pH value 7.8-8.5 where alkalinity is the major problem in this place. The alluvial soil is defined as arid and brown soil or tropical arid brown soil. The Eastern Punjab consist of clayey to loamy soil.

In recent years, heavy metal has become a major concern associated with health and environment related problems. Soil is the vital component of urban and rural environment but due to toxicity caused by heavy metal, soil texture becomes a major concern in regions like Punjab and worldwide. Heavy metal toxicity in the soil leads to change in its physiochemical properties, its fertility and results in the minimum yield of crops grown (Kistan *et al*. 2018).Through food these heavy metals enter inside our body and harm the different body organs such as: liver, kidneys, eyes, lungs etc (Jaishankar *et al.* 2014). In Punjab region it is one of the major causes of cancer (Bangotra *et al.* 2023).

From the physicochemical analysis of soil samples in this study,it is observed that soils had slightly alkaline pH ranges from(7.32 ± 1.25 to 8.21 ± 1.33), electrical conductivity (from 0.26 to 0.32 ds/m). The color of soil samples varied from black to dark brown. Microbes are very important for plants that benefits them in various ways including plant growth and development. PGP microbial isolates are very beneficial for plants having no side effects on host, nearby environment and ecology. In plants, zinc being one of the vital elements, is critical for their growth. However, only minor quantities of zinc are required by plants but,it isvery important because it performs diverse functions in plant such as digestion,regulation, floral development, gametogenesis, reproduction and growth. As a result of zinc deficiency, there is low crop yield of plants and, also, the seeds that are formed contain low concentration of zincwhich can cause poordietary content of zinc in the body after its consumption (Pathak *et al.* 2012). Deficiency of zinc is one of the topmost ten risk factors that is responsible for ailment and demise in the emerging world (Pathak *et al.* 2012). Various studies have revealed the PGP effects of zinc-solubilising microbiota (Gontia*et al.* 2017; Ramesh et al. 2014). In this study, most of the isolates found promising (59.1%) that can solubilize zinc from insoluble zinc compounds. Our study is in agreement with various researchers (Bhakat*et al.* 2021; Mumtaz*et al.* 2017; Negi*et al*. 2022; Yasmin*et al.* 2021). In order to provide the plants with zinc from the soil system, these promising isolates may be considered beneficial.

Phosphorus is considered as another important constituent of the structure of nucleic acid that performs a key role in the protein synthesis regulation, development of newly formed tissue, cell division,storage and energy transfer, photosynthesis, respiration, suitable root development and speeding up the maturity process in plants. To sustain optimum plant production and quality, phosphorus is required as a primary nutrient for plant growth (Zapata and Zaharah 2002). Phosphorus is mainly unavailable for plants because it is present in immobilized form in the soil, thus, by making it available to the plants, thesebacteria that solubilizes phosphate plays a vital role in growth of plant. In our research 50% isolates found able to mobilize complex phosphorous. Deeksha and co-workers also revealed the same (Blanco *et al.* 2020). Our findings are in agreement with (Tian *et al.* 2021; Moreno *etal.* 2021).

Indole acetic acid (IAA) is considered as one of the chiefplant hormones as it performs an importantrole in signaling of plant development, regulation, cell elongation, cell division and cell differentiation (Saeid*et al.* 2018). In this study, it was observed that fifteen isolates (68.1%) found positive for IAA production. This study is in agreement with researchers (Chen *et al.* 2017; Hwang *et al.* 2021).

Since there are many microbes that cause infection to the host plants thus, these PGPB plays an important role in defending the host against the pathogenic attack. Another vital trace element for all living organisms is iron. Siderophore is a high affinity ferric iron chelating compound that is secreted by organisms having low molecular weight (500-1000 da), (Bholay *et al*. 2012). The siderophore production seizes iron in the root environment thus, make it less accessible to compete with harmful microflora (Bagnasco *et al.* 1998; Deshwal 2012). In addition to inhibiting fungal growth by competing for iron, bacteria that produce siderophores also play a role in induced systemic resistance (ISR) in plants, thereby promoting plant growth. Several plant diseases are typically controlled by ISR, a biocontrol method (Niu*et al.* 2016). The plant's immune system gets stimulated thus, making it less vulnerable to pathogenic attack. Siderophores not only form stable complex with heavy metals but also increase the concentration of soluble metals (Datta and Chakrabartty 2014). As a result, it aids in reducing the stress that was imposed by heavy metal-contaminated soil on plants. In this study it is observed that fourteen isolates (63.6%) microbial isolates produced siderophore. The same is also described by researchers (El *et al.* 2019; Létoffé *et al.* 2022).

MicroorganismsproducingHCN have been proposed to perform a key role in the bio-control of pathogens (Jain*et al.* 2020). It is found that cyanogenetic bacteria were known to inhibit the growth of numerous pathogenic fungi,insects, termites and nematodes (Sehrawat *et al.* 2022).Thus by using bacteria that produce HCNas biopesticides is an eco-friendly approach for sustainable agriculture. In the present study, ten microbial isolates(45.4%) werefound positive for HCN production and may be used as a promising biocontrol of plant pathogens. Our study is in agreement with researcher (Lanteigne*et al.* 2012).

Phylogenetic study revealed that the strain N1D9 show sequence similarity of 99.28% with *Pseudomonas putida,* strains N2D4 and N2D6shows sequence similarity 99.17% and 99.02%, strains N3D8 resembles *Bacillus rhizoplanae* showing 99.44% sequencesimilarity.

In wheat variety PBW826, the seeds inoculated with *Pseudomonas* strains N1D9, N2D4, N2D6 have shown 35.3%, 34.3% and 39.3% increase in plantheight, 64.9%,38.7% and 58.1% increase in shoot dry weight, 76.4%, 52.9% and 64.7% increase in root dry weight and 27.2%, 29.1% and 36.3% increase in yield of seed as compared to heavy metal control. Same has been also described by researchers (Jamil*et al.* 2018; Sharma*et al*. 2011). The seeds inoculated with *Bacillus* strain N3D8 increases the plantheight by 36.7%, dry weight of shoot by 59.6%,dry weight of root by 69.1% and 41.8%increase in yield as compared toheavy metal control. Our study is in agreement with (Awan*et al.* 2020; Ayaz*et al.* 2022).

In wheat variety 3086, inoculating the seeds with *Pseudomonas* strains N1D9, N2D4, N2D6, have shown32.1%, 34.9% and 24.8% increase in plant height, 66.2%, 35.3% and 59.6% increase in dry weight of shoot, 50.7%, 77.6% and 67.1% increase in dry weight of root, 27.7%, 29.6% and 33.3% increase in seed yield as compared to heavy metal control. Same has been also described by researchers (Jamil*et al.* 2018; Sharma*et al*. 2011). The seeds inoculated with *Bacillus* strainN3D8 increases 36.3% increase in plant height, 66.2% increase in dry weight of shoot, 71.6% increase indry weight of root and 35.1% increase in seed yield as compared to heavy metal control. Our research is in agreement with (Awan*et al.* 2020; Ayaz*et al.* 2022).

Conclusion

From current study, the isolated strains of *Pseudomonas and Bacillus* exhibits various PGP traits, may be beneficial for plant growth of wheat varieties. Inoculation of wheat varieties PBW826 and 3086 with plant growth increasing microbial isolates enhanced the plant height, dry root weight and dry shoot weight of the crop under heavy metal stress conditions. The results suggested that these isolated microbial strains not only tolerate the heavy metal stress conditions but could enhance the plant growth, thus act as a sustainable biocontrol agent. However, the field trials are required to evaluate the efficacy and contribution of the isolated microbes towards the crop yield and yield related traits under the heavy metal stress conditions.

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List of Tables

Table-1 Molecular identification of isolates

Sr. No.	Strain	Identified as	Similarity percentile	Query conduct	Accession no.

1.	N1D9	*Pseudomonas putida*	99.28%	99%	OQ581751
2.	N2D4	*Pseudomonas protegens*	99.17%	100%	OQ581752
3.	N2D6	*Pseudomonas protegens*	99.02%	99%	OQ581753
4.	N3D8	*Bacillus rhizoplanae*	99.44%	99%	OQ581754

Table-2 Physicochemical Properties of Soil

Sample no.	Soil colour	pH	Electrical conductivity	Organic carbon (%)	Organic matter (%)	Total nitrogen (%)
1.	Black	8.2	0.32	0.42	0.04	0.87
2.	Brown	8.0	0.30	0.46	0.03	0.77
3.	Brown	7.9	0.26	0.49	0.037	0.82
4.	Dark brown	7.3	0.28	0.62	0.033	1.07

Table-3 Heavy metal concentration in soil samples

Sample no.	Heavy metal conc.(mg/kg)	
	As	**Cr**
1.	47	243
2.	56	339
3.	53	295
4.	31	344

Conc.= Concentration; As=Arsenic; Cr= Chromium

Table-4: Colony count after Day 1

Sample No.	Colony Count			
	NAM		**PDA Media**	
	Fungi	**Bacteria**	**Fungi**	**Bacteria**
1.	0	19	0	74
2.	0	1	0	8
3.	0	5	0	20
4.	0	108	0	76

NAM= Nutrient agar media; PDA=Potato dextrose agar media

Table-5 Colony count after Day2

Sample No.	Colony count			
	NAM media		**PDA Media**	
	Fungi	**Bacteria**	**Fungi**	**Bacteria**
1	0	32	1	93
2.	1	7	0	22
3.	1	93	2	34

4.	2	185	2	83

NAM= Nutrient agar media; PDA=Potato dextrose agar media

Table-6 Colony morphology of bacterial isolates

S. No.	Shape of Bacterial Colonies					
	Small regular	**Small irregular**	**Mucoid regular**	**Mucoid irregular**	**Large regular**	**Large irregular**
1.	19	10	8	10	8	5
2.	1	0	4	1	1	0
3.	160	0	4	2	3	2
4.	15	9	20	16	11	18

Table-7 Morphology and Biochemical characteristic features of isolates

Isolates	Gram staining	Indole production test	Methyl red reaction test	Voges-Proskauer test (VP)	Citrate utilization test	Oxidase test	Catalase test	Urease test	Protease test	Motility	Shape
N1D1	-	+	-	-	+	-	+	+	-	+	Rod
N1D2	+	-	-	+	+	-	-	+	-	+	Rod
NID3	-	-	+	-	-	+	+	-	-	+	Rod
NID4	-	+	-	-	+	+	-	-	-	+	Rod
NID5	-	-	+	-	+	+	-	+	+	+	Rod
NID6	-	+		-	-	-	-	+	-	+	Spiral
N1D7	-	+	-	-	+	+	+	-	+	+	Rod
NID8	+	-	-	+	-	-	+	-	-	+	Rod
N1D9	-	+	-	-	+	+	+	+	+	+	Rod
N2D1	-	-	-	+	+	-	-	-	-	+	Rod
N2D2	-	-	-	-	+	+	+	-	+	+	Rod
N2D3	-	-	+	+	-	-	+	-	-	+	Rod
N2D4	-	-	-	-	+	+	+	-	+	+	Rod
N2D5	+	+	-	-	-	-	+	-	-	+	Rod
N2D6	-	-	-	-	+	+	+	-	+	+	Rod
N3D1	+	-	-	+	+	+	+	-	+	+	Rod

N3D2	-	-	-	+	-	-	+	-	-	+	Rod
N3D4	+	-	+	-	-	+	-	-	-	+	Rod
N3D5	+	-	-	+	-	-	-	+	-	-	Cocci
N3D6	-	+	+	-	+	-	-	+	-	+	Rod
N3D7	-	-	-	-	+	+	+	-	+	+	Rod
N3D8	+	-	-	+	+	+	+	-	+	+	Rod

Table-8 Evaluation of PGP traits of selected isolates

Isolates	IAA Test	Phosphate solubilization test	HCN Test	Zinc solubilization test	Chitinase	Siderophore
N1D1	+	-	+	-	-	+
N1D2	+	-	-	-	+	+
NID3	-	+	+	-	-	-
NID4	+	-	-	+	-	-
NID5	+	-	-	+	-	+
NID6	+	-	+	-	-	+
N1D7	-	+	-	+	-	+
NID8	-	-	+	+	-	+
N1D9	+	+	+	+	+	+
N2D1	+	+	-	+	_	-
N2D2	+	-	+	-	+	-
N2D3	-	-	-	+	-	+
N2D4	+	+	+	+	+	+
N2D5	+	-	-	-	+	+
N2D6	+	+	+	+	+	+
N3D1	-	+	+	+	-	-
N3D2	+	+	-	-	+	-
N3D4	-	-	-	+	+	-
N3D5	+	+	-	-	+	-
N3D6	+	-	-	+	+	+
N3D7	-	+	-	-	+	+
N3D8	+	+	+	+	+	+

IAA=Indole acetic acid; HCN=Hydrogen cyanide test; + = positive; - = negative

Table-9 Effect of microbial inoculation on wheat plantheight and weight (PBW-826)

Treatment	Plant height (cm)	Shoot dry weight (gm)	Root dry weight (gm)	Seeds/plant
Control	70.7 ± 2.5	2.19 ± 0.20	0.81 ± 1.1	61
H.M.C.	62.3 ± 1.9	1.91 ± 0.9	0.68 ± 1.2	55

N1D9	84.3 ± 1.5	3.15 ± 0.60	1.20 ± 1.3	70
N2D4	83.7 ± 2.1	2.65 ± 1.2	1.04 ± 1.9	71
N2D6	86.8 ± 1.9	3.02 ± 0.8	1.12 ± 0.9	75
N3D8	85.2 ± 1.7	3.05 ± 0.9	1.15 ± 1.7	78

H.M.C.= Heavy metal control

Table-10 Effect of microbial inoculation on wheat plantlet height and weight (3086)

Treatment	Plant height (cm)	Shoot dry weight (gm)	Root dry weight (gm)	Seeds/plant
Control	68.7 ± 2.4	2.13 ± 0.21	0.79 ± 1.1	60
H.M.C.	60.3 ± 1.8	1.81 ± 0.9	0.67 ± 1.2	54
N1D9	79.7 ± 2.1	3.01 ± 0.6	1.01 ± 1.3	69
N2D4	81.4 ± 2.7	2.45 ± 1.3	1.19 ± 1.9	70
N2D6	75.3 ± 0.9	2.89 ± 0.9	1.12 ± 0.9	72
N3D8	82.2 ± 1.9	3.01 ± 0.94	1.15 ± 1.7	73

H.M.C.= Heavy metal control

List of Figures:

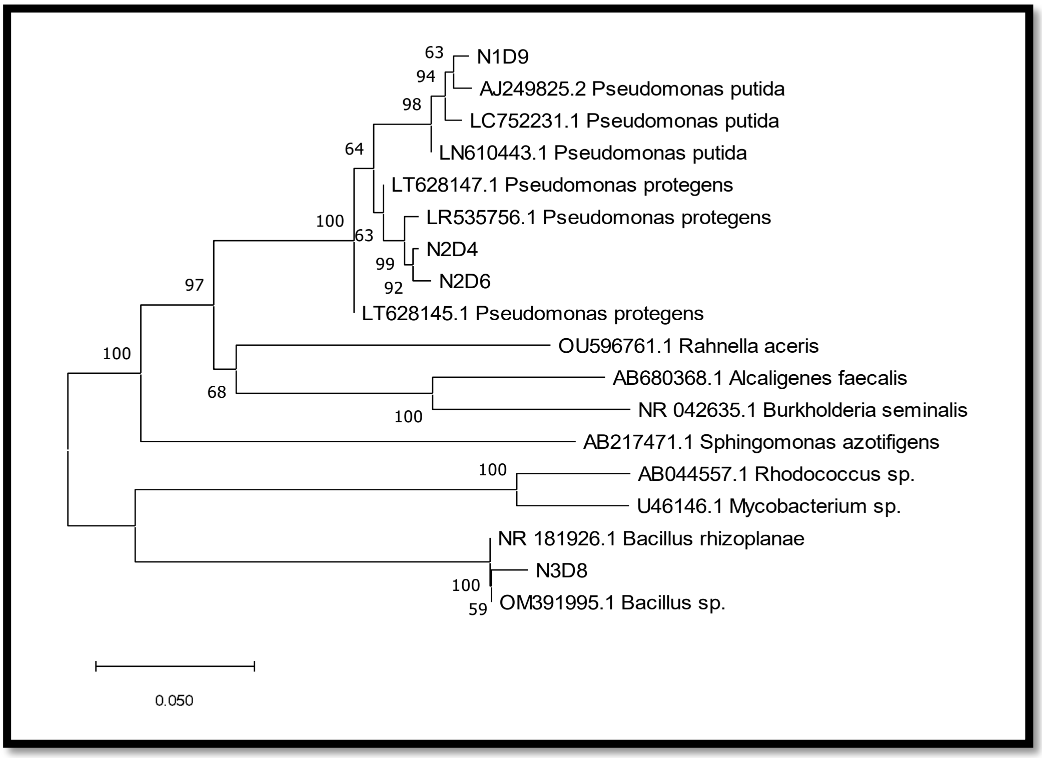

Figure1. Phylogenetic tree (N-J tree) showing the relationship between isolates from this study and other type strains based on ITS region DNA sequences for structure analysis; bootstrap values derived from 1000 replicates.