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Waterborne *Escherichia coli*: Biosafety and Screening as Plant Growth Promoting Rhizobacteria

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Coliforms bacteria are indicator of water portability but they may also harbor beneficial traits for agricultural applications. The present study focused on the detection and biosafety of waterborne non-shiga-toxigenic *Escherichia coli* as plant growth promoting rhizobacteria. Analysis of water samples from different localities of Lahore, Pakistan showed high MPN (most probable number) index that indicated water is not safe for drinking. Nevertheless, *in vitro* screening (especially for auxin production) of non-virulent strains of *E. coli* showed good prospects for crop inoculations. Strains of *E. coli* were identified by biochemical profiling using RapID™ ONE Identification System. Final taxonomic status of strains was further confirmed by 16S rRNA gene sequencing. Biosafety of strains for seed bacterization of *Vigna radiata* (L.) was assessed by evaluating their multidrug resistance pattern and screening for surface antigen O157. Maximum auxin production for strains N-33 (135 μg ml^{-1}), N-40 (127 μg ml^{-1}), N-39 (120 μg ml^{-1}) and N-11 (105 μg ml^{-1}) was recorded in L-tryptophan amended medium. In pot trials (axenic conditions), highly significant improvements for shoot length (up to 43%), number of roots (up to 175%) and biomass (up to 150%) were recorded, over control. Similarly, at full maturity, bacterial inoculations significantly enhanced number of pods (64%) and seed weight (14%). Findings of this study suggested that, after biosafety screening, waterborne *E. coli* has a good prospect to be used as plant growth promoting rhizobacteria.

Key words: Coliforms, *Escherichia coli*, Plant growth promoting rhizobacteria, Most probable number, *In vitro* auxin production.

Total coliforms are a group of bacteria commonly found in water, soil, vegetation as well as in the intestines of mammals including humans. *Escherichia coli* is the member of coliforms and mostly present as mixed microbial populations in contaminated water. Selective or differential media can be used for evaluating *E. coli* levels independent from mixed coliforms population. It is also known that some *E. coli* strains have developed the ability to cause diseases of the gastrointestinal and urinary tract in even healthy people. *E. coli* O157 is an enterohemorrhagic strain of *E. coli* that causes food and water borne illness. It was first reported in 1982 when two outbreaks of bloody diarrhea led to the identification of this new shiga toxin producing *E. coli* O157. This group of enteropathogenic *E. coli* is nowadays termed enterohemorrhagic *E. coli* or EHEC [1, 2, 3].

Recent studies have showed that many bacteria pathogenic to humans and other mammals can use a multiple range of variable hosts. Several human pathogenic bacteria such as *Salmonella enterica*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *E. coli* and *Listeria monocytogenes* are found to have potential to

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infect and colonize animals as well as plants [4, 5, 6]. These pathogenic organisms are the cause of many food and waterborne diseases and transmitted through food chain. Therefore, pathogenic bacteria can contaminate plant surfaces and actively interact and colonize them as an alternate hosts [7]. Besides beneficial bacteria, the components of root exudates also attract potential human pathogens that also evolved to respond different plant signals. In this way, potentially pathogenic strains are supposed to survive and become enriched in the vicinity of the roots where they rapidly utilized simple organic compounds [8].

Numerous bacteria have already been shown to directly stimulate the growth of plants using a variety of mechanisms. They may fix atmospheric nitrogen, synthesize siderophores, production of phytohormones, minerals solubilization, and synthesis of the enzyme 1-aminocyclopropane-1-carboxylic acid (ACC). A bacterium may affect plant growth and development using any one or more of these mechanisms [9, 10, 11, 12, 13]. We propose that the ubiquity of *E. coli* in diverse waters and its significant ability to enhance plant growth clearly suggests that *E. coli* should be treated as crop inoculants for plant growth promotion along with an indicator of the possible presence of other fecal coliforms bacteria. Keeping this in view, we are evaluating the role of waterborne non-toxigenic strains of *E. coli* in enhancing the growth and yield of *Vigna radiata* (L.) Wilczek. To the best of our knowledge, we are first time reporting the detection and biosafety of water borne *E. coli* as plant growth promoting rhizobacteria. For this purpose, potable water samples were collected from different localities of Lahore, Pakistan. Samples were analyzed in terms of total coliforms by most probable number (MPN) and the presence of *E. coli* was confirmed by using selective media. Biosafety of *E. coli* as crop inoculants was confirmed by evaluating their multidrug resistance patterns. Further non-shiga-toxigenic status of *E. coli*; especially, for O157 surface antigen was verified by using Sorbitol MacConkey (SMAC) agar and Prolex™ Latex Agglutination Kit. Hence, in present study, water samples were concurrently assessed for quality and screening for isolated *E. coli* for plant growth promotion.

MATERIALS AND METHODS

Water analysis by most probable number

The basic protocol of Cappuccino and Sherman [14] was used for standard qualitative analysis of water with slight modifications. Potable water samples were collected from different localities of Lahore, Pakistan. Sample collection was carried out in sterile borosilicate bottles and transported to the laboratory for testing within 2 h. The three basic tests i.e. presumptive, confirmed and completed were performed to detect coliforms by means of the most probable number (MPN). Measured aliquots of the water to be tested were added to a lactose broth (LB) in double or single strength. These tests detect the presence of coliforms, gram negative, non-spore forming bacilli that ferment lactose broth with the production of acid and gas. After the presumptive analysis, 40 bacterial strains that showed close resemblance with *E. coli* were selected and streaked on Eosin Methylene Blue (EMB) agar. Finally, 10 bacterial strains (N-3, N-4, N-11, N-16, N-21, N-32, N-33, N-35, N-39 and N-40) that were sorbitol fermenters, negative for surface antigen O157 and positive for *in vitro* auxin production were selected for further study.

Biochemical profiling of bacterial isolates

Strains of *E. coli* were identified by using RapID™ ONE Identification System (Remel, Thermo Scientific). RapID™ ONE System is a qualitative micromethod employing conventional and chromogenic substrates for the identification of *Enterobacteriaceae* and other selected oxidase-negative, gram-negative bacilli. A suspension of the test organism in RapID™ inoculation fluid was prepared to inoculate the panel according to manufacturer's instruction. After incubation, each test cavity was examined for the development of the color. A 7-digit code generated from positive and negative test scores was submitted online at ERIC@ (Electronic RapID™ compendium) for the identification of strains.

16S rRNA gene sequencing

After biochemical profiling, the final taxonomic status of *E. coli* strains was also confirmed by 16S rRNA gene sequencing. Genomic DNA was extracted from overnight grown bacterial cultures by using Genomic DNA Purification Kit

(Promega, Madison, USA). A portion of the 16S rDNA was amplified according to the method described by [15]. About 1·5-kb DNA fragment containing 16S rRNA gene was amplified using forward 27f and reverse primer 1522r [16]. PCR amplification was performed by using 50 µl of Dream Taq™ Green PCR Master Mix (Fermentas) with 0.5 µg of chromosomal DNA template and 0.5 µM of each primer as described earlier [17] After amplification, product was purified using GeneJET Gel Extraction Kit (Thermo Scientific) and sequenced using 27f primer by ABI PRISM-3100 Genetic Analyzer (Applied Biosystems, USA).

Evaluation of biosafety of *E. coli* for crop inoculation

Biosafety of strains before seed bacterization was assessed by evaluating their multidrug resistance pattern and screening for surface antigen O157. Antimicrobial susceptibility test discs (Bioanalyse Co., Ltd., Turkey) were used to evaluate the sensitivity pattern of strains. Sensitivity was determined by using antibiotic discs of amikacin (30 µg), ampicillin (10 µg), amoxicillin (25 µg), cephalexin (30 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), gentamicin (10 µg), norfloxacin (10 µg), tobramycin (10 µg) and nalidixic acid (30 µg). Mueller-Hinton agar was heavily inoculated with bacterial inoculum and antibiotic discs were aseptically transferred to the surface in triplicate. After incubation at 37 °C for 24 h, plates were examined for the presence of zone of inhibition. A measurement of the diameter of the zone in millimeters was made with inhibition zone ruler and compared with standard chart provided by the manufacturer.

To exclude the possibility of enterohemorrhagic *E. coli* O157, strains were grown on SMAC agar. Sorbitol fermentors strains were then screened for somatic antigen O157 using Prolex™ Latex Agglutination Kit (Pro-Lab, Diagnostics). The whole procedure was accomplished according to manufacturer's instructions. For each test strain, fresh colonies were picked from overnight incubated plates and suspended in 0.2 ml normal saline to achieve turbidity equivalent to 2 McFarland Standard. One drop of *E. coli* O157 reagent (latex coated with antibodies) was placed on one of the test circles of agglutination card. Afterwards, one drop of test suspension was added and mixed with sterile mixing sticks and examined for agglutination after 2 min. Isolates that gave negative test results were selected for screening plant growth promoting attributes.

Plant growth promoting traits

Out of 40, 10 strains (N-3, N-4, N-11, N-16, N-21, N-32, N-33, N-35, N-39 and N-40) that were confirmed and sorbitol fermenters and O157 negative were selected to evaluate for *in vitro* auxin production in the presence or absence of L-tryptophan. About 25 ml of L-broth supplemented with 500 µg ml^{-1} L- tryptophan was inoculated with bacterial suspension adjusted to 10^7 CFU ml^{-1}. The flasks were incubated at 37ºC for 72 h at incubator shaker. Auxin concentration in bacterial supernatant was recorded by using Salkowski reagent as mentioned previously [11]. For phosphate solubilization, Pikovskaya's agar medium [18] was prepared and streaked with the bacterial cultures. The plates were incubated at 37ºC for 7 to 10 days. The formation of clearing zones around bacterial growth was taken as positive test for phosphate solubilization. HCN production was determined as described by [19].

Pot trials

Certified seeds of *Vigna radiata* (L.) Wilczek var. NM-92 were procured from Punjab Seed Corporation, Lahore, Pakistan. Seeds were surface sterilized in 0.1% $HgCl_2$ for 1-2 min followed by repeated washings with sterilized distilled water. Seed bacterization was carried out by incubating sterilized seeds in bacterial cell suspension adjusted to 10^7 CFU ml^{-1}. For experiments under axenic conditions, seeds were inoculated in pots containing 100 gm of autoclaved sandy soil. After emergence, 5 uniform seedlings were kept in each pot with six replicates. After 2 weeks, growth parameters in terms of shoot length, root length, numbers of roots, seedlings fresh and dry weight were recorded. The experiments were conducted at 25ºC, 50% humidity and 12 h photoperiod with light intensity of 150-200 µmol m^{-2} s^{-1} in Environmental Test Chamber (MRL-350H; Sanyo, Osaka, Japan). For experiments under natural environmental conditions, bacterial treated seeds were sown in large earthen pots containing 10 kg of unfertilized garden soil as mentioned previously [12]. After germination, seedlings were thinned to 5 per pot with six replications. All pots were arranged in a completely randomized design in the wire house

of Department of Microbiology and Molecular Genetics, University of the Punjab from February to May, 2013 under ambient light and temperature. At full maturity, growth parameters including shoot length, number of pods, and weight of 100 seeds were recorded.

Statistics

Data for bacterial auxin production and plant growth parameters was subjected to analysis of variance (ANOVA) by using SPSS 16 program (SPSS Inc., Chicago, IL). Means of different treatments were separated by using Duncan's multiple range test (P = 0.05).

RESULTS

Detection and characterization of coliforms

In presumptive test, inoculated tubes were examined for gas production. Positive tubes from all three sets were counted to compare with MPN index for the determination of total coliforms in 100 ml water sample (Fig. 1). Positive results were recorded for majority of the samples with MPN index 2400 that indicates water is non potable. Positive tubes from presumptive test were streaked on EMB agar for the detection of *E. coli*. Total 40 *E. coli* strains were isolated and characterized by growing on SMAC agar. Finally, 10 strains that were sorbitol fermenters were selected for further characterization and screening.

Identification of bacterial strains

Isolates were identified by biochemical profiling using RapID™ One Identification System (Table 1). Majority of the strains showed positive results for ornithine, lysine, sorbitol, Á-nitrophenyl-ß,D-galactoside and indole production. On the other hand, none of the strain gave positive result for urease, fatty acid ester,

Table 1. Identification of *E. coli* by RapID™ ONE Identification System

Tests	Strains									
	N-3	N-4	N-11	N-16	N-21	N-32	N-33	N-35	N-39	N-40
URE	-	-	-	-	-	-	-	-	-	-
ADH	+	-	+	+	-	-	-	-	+	-
ODC	+	+	+	+	+	-	+	+	+	+
LDC	+	+	+	+	+	+	+	+	+	+
TET	-	-	-	-	-	+	-	-	-	-
LIP	-	-	-	-	-	-	-	-	-	-
KSF	-	-	-	-	-	-	-	-	-	-
SBL	+	+	+	+	+	-	+	+	+	+
GUR	-	-	+	+	-	+	-	-	-	-
ONPG	+	+	+	+	+	-	-	+	+	+
GLU	-	-	-	-	-	+	-	-	-	-
XYL	-	-	-	-	-	-	-	-	-	-
NAG	-	-	-	-	-	-	-	-	-	-
MAL	-	+	-	-	-	+	-	-	-	-
PRO	-	-	-	-	-	-	-	-	-	-
GGT	-	-	-	-	-	+	-	-	+	-
PYR	-	-	-	-	-	-	-	-	-	-
ADON	-	-	-	-	-	-	-	-	-	-
IND	+	+	+	+	+	+	+	+	+	+
RapID™ Code	6121 001	4121 201	6121 001	6121 001	4121 001	4121 111	4120 001	4121 001	6121 011	4121 001

Similarity>99% with *Escherichia coli*

Abbreviations: URE, Urea; ADH, Arginine; ODC, Ornithine; LDC, Lysine; TET, Aliphatic thiol; LIP, Fatty acid ester; KSF, Sugar aldehyde; SBL, Sorbitol; GUR, ρ-Nitrophenyl-β,D-glucuronide; ONPG, ρ-Nitrophenyl-β,D-galactoside; GUL, ρ-Nitrophenyl-β,D-glucoside; XYL, ρ-Nitrophenyl-β,D-xyloside; NAG, ρ-Nitrophenyl-n-acetyl-β,D-glucosaminide; MAL, Malonate; PRO, Proline-β-naphthylamide; GGT,γ-glutamyl-β-naphthylamide; PRY, Pyrrolidonyl-β-naphthylamide; ADON, Adonitol; IND, Trytophan

sugar aldehyde, Á-nitrophenyl-ß,D-xyloside, Á-nitrophenyl-n-acetyl-ß,D-glucosaminide, pyrrolidonlyl-ß-naphthylamide and adonitol. A 7-digit microcodes obtained from the scoring of positive tests were submitted at ERIC® (http://www.remel.com/ERIC/IdentificationSingle.aspx) for the identification of the test organisms. After comparison, strains showed more than 99% similarity with *E. coli*. After identification with RapID™ System, sequences of 16S rRNA gene were compared to online database (GenBank) through BLAST to confirm the final taxonomic status of isolates. The sequences of *E. coli* strains have been deposited in the GenBank under accession numbers KJ154049 to KJ154058.

Biosafety of *E. coli* as plant growth promoting rhizobacteria

Biosafety of strains as PGPR was confirmed by evaluating the multidrug resistance pattern and screening for the presence of somatic antigen O157. Disc diffusion method was used to evaluate the antibiotic sensitivity of *E. coli* (Table 2). Results showed that majority of the strains were sensitive against amikacin, ciprofloxacin,

Table 2. Antibiotic susceptibility pattern of *E. coli* isolated from potable water sample

Strains	Antibiotics									
	Ami (30 µg)	Amp (10 µg)	Amo (25 µg)	Cep (30 µg)	Cip (5 µg)	Chl (30 µg)	Gen (10 µg)	Nor (10 µg)	Tob (10 µg)	Nal (30 µg)
	Susceptibility (Zone size in mm)[a]									
N-3	S (20)	S (17)	I (13)	S (18)	S (25)	S (18)	S (18)	S (24)	S (18)	R (08)
N-4	S (18)	S (20)	I (15)	S (16)	S (30)	S (20)	S (16)	S (33)	S (20)	S (19)
N-11	S (23)	I (15)	S (22)	S (17)	S (33)	S (25)	S (21)	S (27)	R (10)	I (18)
N-16	S (19)	S (18)	I (17)	S (20)	S (26)	I (13)	S (25)	S (25)	R (08)	S (22)
N-21	S (23)	R (07)	S (20)	I (15)	S (28)	S (30)	S (28)	S (22)	R (11)	S (24)
N-32	S (20)	R (03)	S (21)	S (21)	S (26)	S (24)	S (17)	S (21)	S (22)	S (20)
N-33	S (24)	I (16)	S (19)	S (18)	S (32)	R (08)	S (22)	S (28)	S (21)	R (06)
N-35	S (21)	R (04)	I (14)	S (19)	S (29)	S (22)	S (26)	S (30)	I (13)	S (19)
N-39	S (19)	R (03)	R (08)	S (18)	S (25)	S (20)	S (25)	S (22)	S (17)	S (21)
N-40	S (22)	I (16)	S (20)	S (07)	S (27)	S (18)	S (20)	S (26)	S (15)	S (22)

Abbreviations: Ami, Amikacin; Amp, Ampicillin; Amo, amoxicillin; Cep, Cephalexin; Cip, Ciprofloxacin; Chl, Chloramphenicol; Gen, Gentamicin; Nor, Norfloxacin; Tob, Tobramycin; Nal, Nalidixic acid; S, Sensitive; I, Intermediate; R, Resistant

[a] Values in parenthesis indicate zone of inhibition

Table 3. Effect of *E. coli* on vegetative growth of *V. radiata* (L.) under axenic conditions

Strains	Shoot length (cm)	Root length (cm)	No of roots	Fresh weight(g)	Dry weight (g)
Control	17.20 (a)	5.00 (a)	4.00 (a)	2.26 (a)	0.40 (a)
N-3	18.40 (ab)	5.50 (a)	9.00 (cd)	2.50 (a)	0.45 (a)
N-4	21.00 (bcd)	5.20 (a)	6.00 (abc)	3.20 (ab)	0.80 (bc)
N-11	22.40 (cd)	6.20 (ab)	11.0 (d)	4.50 (d)	0.65 (ab)
N-16	21.70 (bcd)	9.20 (c)	9.0 (cd)	4.10 (cd)	0.70 (b)
N-21	21.30 (bcd)	6.55 (abc)	4.0 (a)	3.40 (bc)	0.55 (ab)
N-32	19.10 (abc)	7.20 (abc)	10.0 (d)	3.90 (cd)	0.43 (a)
N-33	24.60 (d)	6.40 (ab)	9.00 (cd)	3.70 (bc)	0.60 (ab)
N-35	21.30 (bcd)	7.85 (abc)	8.00 (bcd)	3.75 (bc)	1.00 (c)
N-39	22.00 (cd)	8.50 (abc)	9.00 (cd)	2.90 (ab)	0.60 (ab)
N-40	20.95 (cd)	5.85 (ab)	5.00 (ab)	3.20 (ab)	0.50 (a)

Mean of 20 plants. Different letters in parenthesis within the same column indicate significant difference between treatments using Duncan's multiple range test (P= 0.05).

gentamicin and norfloxacin with maximum zone of inhibition of 24, 21, 28 and 28 mm, respectively. For ampicillin, cephalexin, choloramphenicol and tobramycin and nalidixic acid strains were either sensitive or showed intermediate resistance. The detection of somatic antigen O157 was carried out by Prolex™ Latex Agglutination Kit. All biochemically characterized sorbitol positive *E. coli* strains were confirmed negative for O157 (Fig. 2).

Plant growth promoting traits

After evaluating the biosafety concerns, bacterial strains were screened for *in vitro* auxin, phosphate solubilization and HCN production. Bacterial strains N-33, N-39 and N-11 recorded 23, 18 and 17 µg ml^{-1} auxin in the absence of L-tryptophan. However, when medium was supplemented with precursor auxin production was enhanced several folds (Fig. 3). For instance, N-33

Table 4. Effect of *E. coli* inoculations on vegetative and yield parameters of *V. radiata* (L.) at final harvest

Strains	Shoot Length (cm)	Number of pods/ Plant	Weight of 100 seeds (g)
Control	16.30 (a)	13.60 (ab)	3.70 (ab)
N-3	17.00 (ab)	16.00 (abc)	3.83 (abc)
N-4	20.00 (abc)	18.70 (abc)	4.00 (bc)
N-11	19.00 (abc)	22.30 (c)	4.20 (c)
N-16	19.30 (abc)	17.60 (abc)	3.90 (abc)
N-21	20.70 (abc)	18.00 (abc)	3.50 (a)
N-32	16.60 (a)	20.00 (bc)	3.50 (a)
N-33	25.00 (c)	18.60 (abc)	4.20 (c)
N-35	20.30 (abc)	13.00 (a)	3.95 (bc)
N-39	22.30 (abc)	15.00 (ab)	4.10 (bc)
N-40	23.00 (bc)	12.30 (a)	4.00 (bc)

Mean of 20 plants. Different letters in parenthesis within the same column indicate significant difference between treatments using Duncan's multiple range test (P= 0.05)

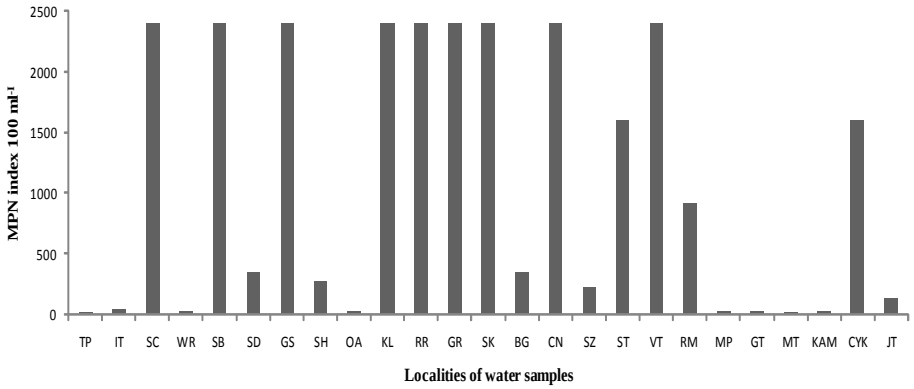

Fig 1. MPN index for coliform bacteria per 100 ml water sample. **Abbreviations:** TP, Taj Pura; IT, Iqbal Town; SC, Sadar Cantt; WR, Wahdat Road; SB, Shad Bagh; SD, Samanabad; GS, Garhi Shahu; SH, Shahdra; OA, Old Anarkali; KL, Kot Lakhpat; RR, Ravi Road; GR, Gulshan Ravi; SK, Shera Kot; BG, Bhati Gate; CN, Chung; SZ, Sabzazar; ST, Shalimar Town; VT, Valentia Town; RM, Rang Mahal; MP, Mughal Pura; GT, Garden Town; MT, Model Town; KAM, Kot Abdul Malik; CYK, Chowk Yateem Khana; JT, Johar Town.

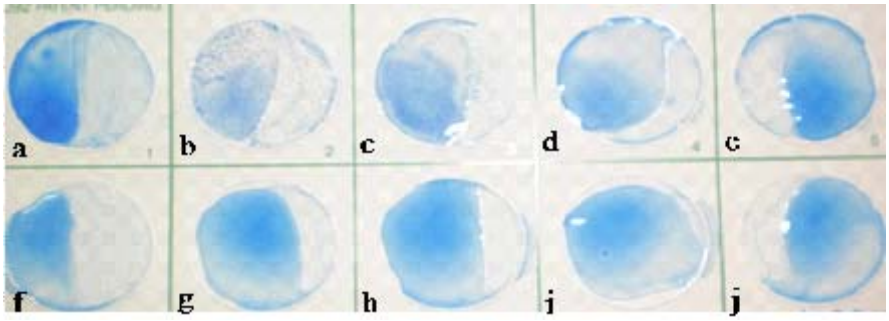

Fig 2. Agglutination reactions of *E. coli* strains. (a)- Negative control, (b)-positive control, (c)- N-3, (d)- N-4, (e)- N-11, (f)- N-21, (g)-N-32, (h)- N-33, (i)- N-35, (j)- N-40

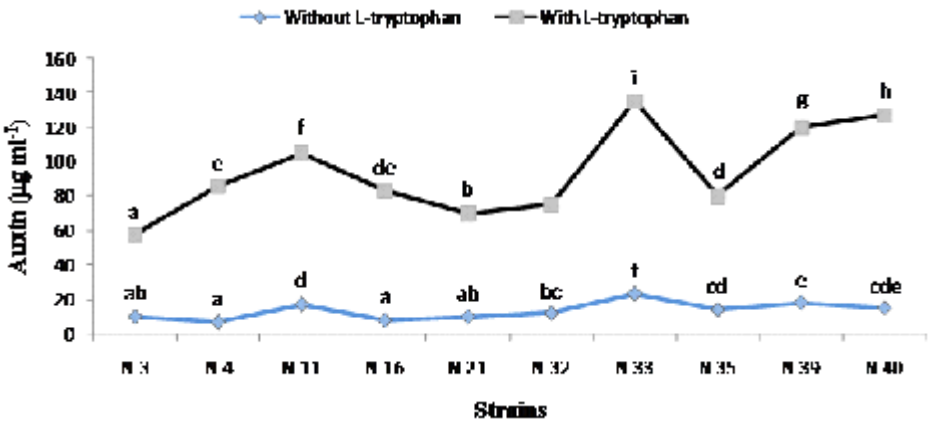

Fig 3. Auxin production by different strains of *E. coli* in the presence and absence of L-tryptophan. Letters at different points indicate significant difference between treatments using Duncan's multiple range test (P = 0.05).

(135 µg ml^{-1}), N-40 (127 µg ml^{-1}), N-39 (120 µg ml^{-1}) and N-11 (105 µg ml^{-1}) showed significant increases as compared to their respective un-supplemented treatments. For phosphate solubilization, none of the strains gave positive results. Nevertheless, N-4, N-33, N-35 and N-40 showed positive results for HCN.

Plant-bacteria experiments

Effect of *E. coli* inoculations on growth of *V. radiata* (L.) was evaluated under axenic and natural wire house conditions. Under axenic conditions, N-33, N-11 and N-39 enhanced shoot length 43%, 30% and 28%, respectively, over water treated control (Table 3). For root length, majority of the treatments showed comparable results to that of control except N-16 that recorded 84 % increment. For number of roots, maximum increases were recorded with N-11 (175%), N-32 (150%), N-3 (125%), N-16 (125%), N-33 (125%) and N-39 (125%). For fresh weight, significant improvements of 100%, 82%, 72%, 66% and 64% were observed for N-11, N-16, N-32, N-35 and N-33, respectively. For dry weight, N-35 (150%) and N-16 (75%) were the most promising as compared to other treatments. Experiments conducted under natural wire house conditions showed variable responses for vegetative and yield parameters (Table 4). For shoot length, significant improvements of 54%, 41%, and 37% were shown by N-33, N-40 and N-39, respectively. For number of pods, majority of the treatments showed statistically comparable results; however, N-11 showed 64% enhancement, over control. In case of seed weight, a maximum increase of 14% was observed with N-11 and N-33.

DISCUSSION

The concentration of fecal coliforms in water is measured to determine the likelihood of contamination by microorganisms. Fecal coliforms are not pathogen but are commonly found alongside pathogenic microbes that are responsible for different types of waterborne illnesses. It is comparatively easier to test for coliforms than for pathogenic microbes; therefore, the presence of coliforms in drinking water is used to indicate potential contamination. The accepted standards for drinking water are that there should be no coliforms present after the water is filtered or processed. In present study, analysis of potable water samples from different localities of Lahore, Pakistan indicated that water is unsuitable for drinking. It is evident from the highest MPN per 100 ml for majority of the water samples (Fig. 1). After water analysis, strains that showed close resemblance with *E. coli* were selected to screen for *in vitro* auxin production. To the best of our knowledge, we are first time reporting the water quality assessment as well as biosafety of waterborne non-shiga-toxigenic *E. coli* as plant growth promoting rhizobacteria.

Taxonomic status of isolates was confirmed by RapID^{TM} One System and 16S rRNA gene sequencing that showed close similarity of strains with *E. coli*. *In vitro* screening revealed that strains of waterborne *E. coli* were showing plant growth promoting traits especially auxin production. For instance, quantity of auxin ranges from 58 to 135 µg ml^{-1} in L-tryptophan amended

medium (Fig. 3). This concentration of auxin was comparable or even higher than rhizobacteria that we previously isolated from natural plant settings [11, 12, 20]. The presence of growth promoting traits signifies the potential of *E. coli* to be used as crop inoculants. However, before setting bacteria-plant experiments, biosafety of strains against multiple drug resistance and for the presence of surface antigen O157 was evaluated. Experiments showed that strains exhibited promising growth responses under axenic and natural environmental conditions. Under axenic conditions, highly significant increases for shoot length (up to 43%), number of roots (up to 175%) and biomass (up to 150%) were recorded. Similarly, under natural conditions, bacterial inoculations significantly stimulated vegetative and yield parameters (Table 4). The colonization of rhizosphere of agronomically important crops by opportunistic human pathogens has been reported by different workers [7, 21, 22]. Moreover, in previous studies, these bacteria have also been show to exhibit different plant growth promoting traits including IAA [8, 11, 23]. Recently, it has also been demonstrated that chances of preharvest contamination of grains with enteric pathogens are very low from biosolid amended soil [24].

Findings of the present study suggested that majority of the water samples collected from different localities were not potable; nevertheless, it is a reservoir to isolate non-shiga-toxigenic strains of *E. coli* for agricultural applications. Multiple drug resistance pattern and screening for O157 surface antigen indicated that strains are safe for seed bacterization. Strains also showed significant potential to produce *in vitro* auxin in L-tryptophan amended medium that is comparable with rhizobacteria previously isolated from natural plant settings. Biofertilization potential of strains is evident from growth stimulation of *V. radiata* under axenic and natural environment. Hence, after biosafety screening, waterborne *E. coli* has a good prospect to be used as plant growth promoting rhizobacteria.

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