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Isolation and characterization of *Escherichia coli* from rainwater and its importance in agricultural practices

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Abstract
Global gross domestic products directly dependent on agriculture sector. It has estimated the world population rise up to 9.5 million by 2050, leading to high food consumption. Environmental conditions, biological health of soil and new emerging technologies in food industries are important criteria for achieving the targeted goal of food security. This research is focused to isolate *E. coli* from road side rain water and validation of *E. coli* in different area for substantial progress. *Escherichia* (*E. coli* is a faecal microbe that inhabits the intestines of endotherms (primary habitat) and the natural environment (secondary habitats). Due to prevailing thinking regarding the limited capacity of *E. coli* to survive in the environment, relatively few published investigations exist regarding environmental factors influencing *E. coli*'s survival. To help future researchers, an overview of factors known to impact the survival of *E. coli* in the environment and its interaction with various developmental process is provided. Notably, the lack of historic field-based research holds two important implications: (1) large knowledge gaps regarding environmental factors influencing *E. coli*'s survival in the environment exist; and (2) the efficacy of implemented abiotic stress management strategies has rarely been assessed on larger field scales, thus leaving their actual impact(s) largely unknown. Moreover, the persistence of *E. coli* in the environment calls into question its widespread and frequent use as a faecal indicator microorganism. To address these shortcomings, future work should include more field-based studies, occurring in diverse physiographical regions and over larger spatial extents. This information will provide scientists and land-use managers with a new understanding regarding environmental factors influenced by *E. coli* in plant, thereby providing insight to address problematic faecal contamination effectively.

Keywords: Bacteria, land-use practices, environmental persistence, PGPR

1. Introduction
E. coli are harmless commensal bacteria, some strains can cause human diseases. Shiga toxin-producing *E. coli* (STEC), including enterohemorrhagic *E. coli* (EHEC), can cause bloody diarrhea as well as potentially fatal human diseases, such as hemolytic uremic syndrome (HUS) and hemorrhagic colitis (HC)75). *E. coli* O157:H7 is among the most recognized serotypes of EHEC, and has caused many large outbreaks of food- and water-borne illness. In addition to STEC and EHEC, at least five additional pathogroups of *E. coli* have been identified. Enteropathogenic *E. coli* (EPEC) are one of the major causes of watery diarrhea in infants, especially in developing countries. Enterotoxigenic *E. coli* (ETEC) are the main cause of traveler's diarrhea and enteroaggregative *E. coli* (EAEC) can cause persistent diarrhea, lasting for more than two weeks. Enteroinvasive *E. coli* (EIEC) are genetically, biochemically, and pathogenically closely related to Shigella (Nataro *et al*.,1998, Pupo *et al*.,2000) [29, 35].
Several, researchers consider Shigella as being a subgroup of *E. Coli* (Pupo *et al*.,2000) [35]. While extraintestinal pathogenic *E. coli* (ExPEC), including uropathogenic and avian pathogenic strains, are thought to be harmless while they are in the intestinal tracts, they can cause neonatal meningitis/sepsis and urinary tract infections if acquired by others (Welch *et al*.,2002) [44]. Extensive reviews are available on the pathogenesis, diagnosis, and sources of pathogenic *E. Coli* (Paton *et al*.,1998) [34]. However, the distribution of pathogenic *E. coli* in the environment has not been examined in detail. Several studies have shown that EPEC strains can be more frequently detected in the environment than the STEC (Ishii *et al*.,2007, Lauker *et al*.,2003) [8]. Ishii [8] *et al*. and Lauker *et al.* [11] reported the occurrence of potential EPEC strains, but no STEC, at Great Lake beaches. The broad distribution of potential EPEC in a large number of animal hosts may, in part, explain the frequent detection of this pathogen in the environment.

Diversity of *E. coli*

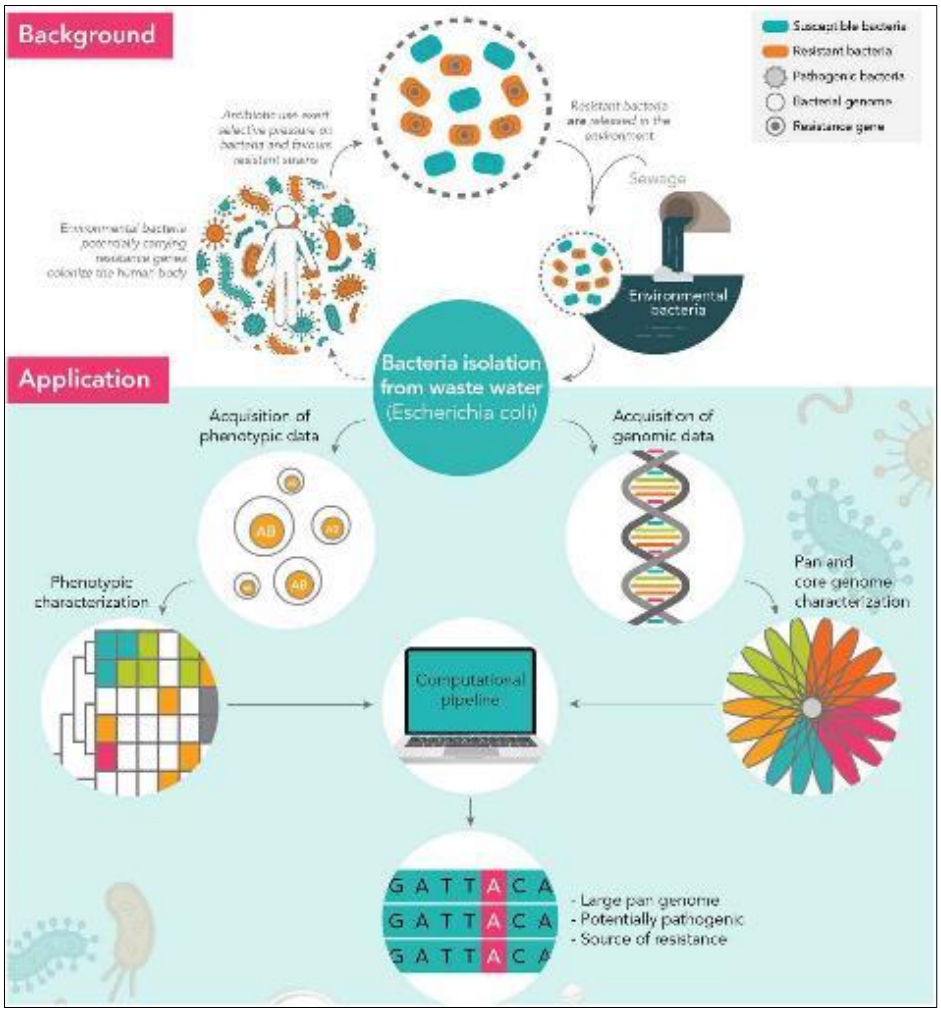

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Fig 1: Flow diagram of isolation of *E. coli* from waste water treatment.

E. coli is genotypically and phenotypically diverse. Traditional classification of *E. coli* is made based on reaction of antibodies with three types of antigens: the somatic (O), capsular (K), and flagellar (H) antigens (Nataro *et al*.,1998) [29] Currently, *E. coli* has been shown to possess 173 O, 103 K, and 56 H antigens, and the number of newly discovered antigens is increasing (The *E. coli* Index [http://ecoli.bham.ac.uk/]). Diverse *E. coli* serotypes, which are defined by the combination of O and H antigens, have been identified. For example, *E. coli* O157:H7 is the most well-known serotype that can cause human disease. *E. coli* strains also vary in other phenotypic characteristics, such as carbon utilization patterns, antibiotic resistance profiles, flagellar motility, ability to form biofilms, and the ability to cause diseases (Anderson *et al*.,2006, Yang *et al*.,2004) [2, 50]. This is probably due to gene mutations and acquisition of new genes via plasmid- or phage-mediated horizontal gene transfer. Genome sequencing has revealed that horizontal gene transfer plays a significant and important role in gene acquisition in *E. Coli* (Welch *et al*., 2002) [44]. Similarly, auxotrophic mutants (*i.e.* mutants that cannot synthesize necessary amino acids for growth) were often obtained from biofilm communities (Cooper *et al*.,2005) [9]. Diversity of *E. coli* is observed at the genotype level as well. While more than 650 genotypes were observed among 1,535 unique *E. coli* strains based on repetitive element palindromic (rep)-PCR DNA fingerprinting, rarefaction analysis revealed that the diversity observed was not saturated. DNA fingerprint patterns are variable even within the same serotype. For example, pulsed-field gel electrophoresis (PFGE) DNA fingerprint patterns of 1,798 *E. coli* strains belonging to the O157 serogroup were only 10% similar (Ohinishi *et al*.,2002). Other factors, such as recombination, can also contribute to genotypic diversity. Recent progress in genome sequencing revealed differences in gene content among *E. coli* strains. The complete genomes of eight *E. coli* strains have been published, including nonpathogenic *E. coli* K12 strains (Blatter *et al*.,2006, Mori *et al*.,2000) [23], EHEC O157:H7 strains, uropathogenic strains (Welch *et al*.,2002) [44], While *E. coli* has diverse genotypic and phenotypic characteristics, some characters are shared among strains exposed to similar environments. If some of the characteristics among *E. coli* strains can be grouped by origin of isolation (*i.e.* host animals, water or soil), then it is possible to use these phenotypes or genotypes as a tool to determine the source of unknown bacteria. This approach is called microbial source tracking (MST), and is discussed below in more detail.

Microbial source tracking

Potential sources of fecal contamination in water, soil, and sediments include human sewage, pets, farm animals, wildlife, and waterfowl. Although recreational beaches are

routinely monitored for the levels of fecal indicator bacteria, microbial numbers alone cannot determine the potential sources of these bacteria. The identification of potential sources of *E. coli* and other fecal indicator organisms (such as enterococci and Bacteroides) in the environment is of great interest to the public, government regulatory agencies, beach managers, and operators of sewage treatment facilities. MST data can be used to establish proper risk assessment and abatement procedures (Stoeckel *et al*.,2007) [43]. Several library-dependent and -independent methods have been developed for MST studies. A library for MST studies contains a dataset of characteristics of the target microorganism from known-source hosts (Stewart *et al*.,2007) [42]. Both phenotypic (e.g. antibiotic resistance profile, carbon utilization patterns) and genotypic characteristics (e.g. DNA fingerprint patterns) can be used for library-dependent MST methods (Krumperman *et al*., 1983, Stewart *et al*., 2007, Stoeckel *et al*.,2007) [20, 43, 42]. Among these, rep- PCR DNA fingerprinting, including horizontal fluorophore enhanced rep-PCR (HFERP) DNA fingerprinting, has been frequently used as a library-dependent MST method. The technique is reproducible, relatively inexpensive to use, and has relatively high throughput as compared to other molecular methods. Several studies have shown that the HFERP DNA fingerprint patterns of *E. coli* strains could be clustered by animal host groups. This indicates that some level of host specificity exists in *E. coli* population. However, when *E. coli* is used as a target organism for MST studies, a large database is necessary to adequately represent diverse genetic and phenotypic characteristics in *E. coli* populations obtained from multiple hosts (Jenkins *et al*., 2003) [19]. Moreover, since *E. coli* is not evenly distributed among host animal species, soil n water, the distribution of this bacterium in the environment is patchy (Byappanahalli *et al*.,2006, Ishii *et al*., 2007) [8]. The distribution of *E. coli* is also subject to geographical and temporal variability, thus adequate care must be taken in obtaining representative samples for the construction and analysis of libraries. Host-specific markers, targeting 16S rDNA and other genes, have been identified for *E. coli*, methanogens, viruses and coliphage, member of the Bacteriodales (Dick *et al*., 2005, Okabe *et al*.,2007, Savichtehava *et al*., 2007) [10, 38, 32], and metagenomic DNA fragments (Shanks *et al*.,2006, Shanks *et al*.,2007) [39, 40]. However, before use in field studies these host-specific markers need to be validated by estimating the proportion of false-positives and false-negatives in the target population, and for sensitivity in detecting these bacteria that are present in low numbers in complex matrices, such as soil and sediment. In some cases, the primers work well when tested with fecal samples, but have sensitivity issues when used with environmental samples, although only a relatively few field investigations have been done using library-independent approaches (Okabe *et al*.,2007, Savichtehava *et al*.,2007, Shanks *et al*.,2006) [38, 32, 39], this method appears to be promising for future MST studies. While *E. coli* is often used as an organism for both library dependent and -independent MST studies, and as a metric for fecal contamination, some researchers criticize its use in MST studies postulating that this bacterium may not be distinct enough to be separated into host source-specific groups. Gordon and Lee (Gordon *et al*.,1999) [14] used multilocus enzyme electrophoresis to characterize enteric bacteria and found that only 6% of the genetic diversity in *E. coli* could be attributed to host animals in Australia. Other studies have shown that while the relationship between E coli genotypes and animal source groups is not perfectly correlated, there is significant clustering of strains by animal or origin. In order to establish a reliable MST method, Malakof suggested that population genetic studies done using more sensitive and discriminative methods are needed to better understand the relationship between diversity and host specificity in *E. coli.*

1.5 Environmental stress management

A wide repertoire of genes/pathways are known to regulate the stress response in *E. coli*, and these include (*a*) the generalised stress response (RpoS), which facilitates survival against nutrient derivation, and oxidative and osmotic stressors (Battesti *et al*., 2011; White *et al*., 2011) [5, 47]; (*b*) the universal stress proteins, known to be important for resistance to oxidative stress, ultraviolet (UV) radiation and antibiotics (Gustavsson *et al*., 2002; Nachin *et al*., 2005) [27]; and (*c*) the more recently discovered locus of heat resistance – a genomic island encoding up to 16 different proteins associated with heat shock, cell envelop maintenance and turnover of misfolded proteins (Mercer *et al*., 2015) [24] – important for repairing cellular damage. Surprisingly, naturalised waste water strains of *E. coli* possess all these stress-adaptive mechanisms (Zhi *et al*., 2016b) [51], which in themselves, are reflective of the processes used to treat waste water (i.e. osmotic shock, oxidative stress (hydrogen peroxide (H_2O_2), chlorine), UV treatment, nutrient deprivation/competition), suggesting an evolutionary adaptation of *E. coli* to this non-host environment.

A further adaptation appears to be the site-specific insertion of the *IS30* element between the *uspC* and *flhDC* genes. Insertion elements located in IGRs have been shown to modify expression of certain genes (Barker *et al*., 2004) [4]. As outlined earlier, the *uspC* gene plays an important role in promoting resistance to oxidative and UV damage through DNA repair (Gustavsson *et al*., 2002; Kvint *et al*., 2003; Nachin *et al*., 2005) [27], suggesting that the *IS30* element may play a role in upregulating *uspC* expression during UV treatment of waste water. The *flhDC* gene encodes the master regulator for flagellar biosynthesis, acting as an activator for the expression of bacterial flagellar proteins (Anderson *et al*., 2010) [2]. Flagella promote bacterial motility, which, in turn, is positively associated with biofilm formation (Wood *et al*., 2006) [1]. Biofilm formation is an important strategy used by bacteria to survive unfavourable environmental conditions, and the formation of biofilms has been shown to promote resistance to chlorine, UV radiation (UVC), oxidative damage and even predation (Vogeleer *et al*., 2014) – important microbial reduction strategies used during waste water treatment. Consequently, the *uspC–IS30–flhDC* polymorphism may play an important adaptive strategy in these naturalised bacterial populations towards colonisation and survival in tertiary treated waste water.

2. Material and methods

2.1 Sampling and Isolation

Samples collected in July from a semiarid region of the Kota, Rajasthan locality (outside road of Modi Institute of Science and Technology college Rajasthan). The average annual temperature is 26.7 ºC to 45ºC.The area is characterized by a semi-arid climate with annual average rainfall ranging from

762 mm). The greatest amount of precipitation occurs in July, with an average of 294mm. Kota is in northern hemisphere. The soil was classified as a typical cinnamon-coloured soil.

The study was conducted by collecting water sample from road side rain water sources. Finally, the collected water samples were processed to analyse the E. coli isolation and identification. Water samples were collected from three rain water sites. During sampling, the rain water as sources were chosen. Selected sites were 100m-120m away from each other. The samples were collected sceptically and kept in ice box in a plastic container during transportation. The samples collected from rain water from road side were serial diluted, lactose broth, plate count and selective media (EMB) were used to detect and isolate total coliform (E. coli) from the three water samples the road side rain water outside the Modi College of Science and Technology premises. These samples and the appropriate dilutions were spread onto different isolation media as follows: EMB Agars, Plates were incubated at 28±2°C, and a representative colony was picked and transferred to fresh EMB agar medium for further studies.

2.2 Sample Analysis

In the laboratory, the three sample from each site were subjected to serial dilution for analysis of E. coli. after each sample were serially diluted in seven test tubes (dilution 10^{-7}), one ml of water from each dilution were aseptically transferred to prepared seven lactose broth test tubes for detection of *E. coli* (coliform) presence.

The prepared tubes were incubated for 48hrs at 37ºC upon completion of the incubation period,

the positive test tubes (tubes with bubble gas formed) counted. From each positive test tubes, the samples were aseptically transferred to selective *E. coli* media for further culturing and identifying it and incubated for 24hrs at 37ºC, upon completion of incubation period, the colony of each Petridish were counted and recorded. Further identification was done by examining the colonies by gram staining technique and observed under 100 x electron microscope (Güldemann, 2000).

3. Result and discussion

3.1 Bacteriological Analysis

Bacteriological analysis of samples from the three sites at Modi Institute of Science and Technology main campus showed that all samples were positive for *Escherichia coli.* This indicator bacterium was often encounter in all samples from water sources of the study area.

The morphological tests were done in aseptic conditions in Laminar air flow (Fig. A). The Gram staining is the test that's always the first step in the identification of a bacterial organism. In gram staining procedure, the isolated bacteria performed gram negative as well as small rod shaped and pinkish colour (Fig. B).

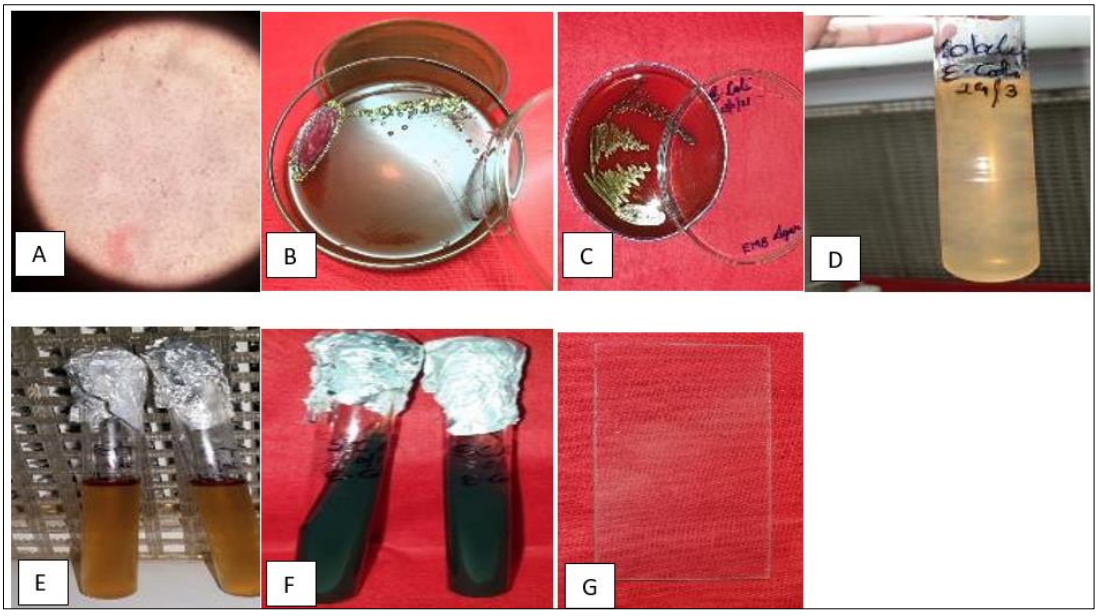

Fig 2: E. *coli* Gram negative strain (Fig. A). *E. coli* mixed colonies on EMB (Fig. B), Metallic Green Sheen colonies of *E. coli,* (Fig. C), Motility test +ve for *E. coli* (Fig. D), Indole test +ve for *E. coli,* (Fig. E), Simmon's Citrate test -ve for *E. coli,* (Fig. F), Catalase test negative for *E. coli* (Fig. G).

The sample was streaked on EMB Agar medium and further was incubated at 37^0C for 24hrs (Fig B). A single colony was picked up and re-cultured a few times on EMB Agar to obtain a pure culture (Fig. C).

The isolated bacteria also showed similar results by different biochemical test. In biochemical test, the motility test indicates, the isolated bacterium was motile, because growth area formation extending away from the inoculation line (Fig. D). In SIM medium test the isolated bacteria was motile, and sulphide production positive because the bacteria produced red/pink colour band on the top of tube when adding Kovac's reagent, Indole test positive (Fig. E) and H_2S did not produced as no black precipitation formed.

Simmons's citrate test was negative because the medium did not turn from green to the dark blue colour (Fig. F). Catalase test demonstrate the absence of catalase, an enzyme that catalyses the release of oxygen from hydrogen peroxide (H_2O_2). In this research we found when we added H_2O_2, to our isolated bacteria did not produce air bubbles that clearly indicates it was negative to catalase (Fig. G).

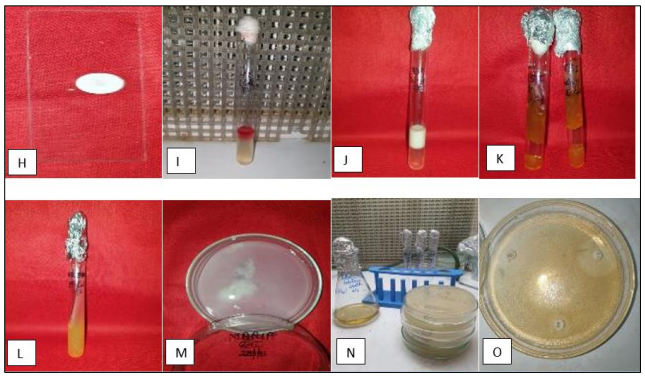

Fig 3: Oxidase Test -ve for *E.coli*, (Fig. H),MR Test +ve for *E.coli.*, (Fig. I), VP Test -ve for *E.coli*, (Fig. J) Triple sugar iron test +ve for *E. coli*, (Fig. K) Urease test +ve for *E. coli*, (Fig. L) *Phosphate solubalization test -ve for E.coli*, (Fig. M) *Nitrate test -ve for E.coli* (Fig.N), Plate *E. coli* A (with antibiotis discs LE,NX,Gf (Fig. O).

In oxidase test, there was no purple colour after inoculated on the oxidation disc, hence it is Negative for oxidase test (Fig. H). After inoculation of the bacteria into Methyl Red medium it produced acid and gives pink colour. So isolated bacteria showed positive result, because it turns a pink colour (Fig. I). In TSI test, isolated bacterium produced gas and hydrogen sulphide and glucose, lactose and or sucrose fermenting because acidic slant and yellow colour were found in medium (Fig. K). In Urease test, isolated bacteria hydrolysed urea, hence it is a Urease positive for *E. coli*, (Fig. L), in Nitrate reductase test the colour did not change to red after addition of sulphanalic acid (Fig. N), hence negative test for *E. coli.* For phosphate solubilizing activity which not seen on NBRIP media after adding tri calcium phosphate, no halo zones were formed after incubation of bacterial isolates for 7 days, Negative test for *E. coli.* (Fig. M). Sentivity test was +ve for *E. coli* (O).

Different test results are summarized in Table 1 Biochemical characteristic of isolated bacteria gram stain, Motility test, Citrate utilization test, Methyl red test, Triple sugar iron test, VP test, Indole test, Urease test, Oxidase test, Catalase test, Phosphate solubilization test and Nitrate reductase test etc.

Table 1: Response of the isolated bacteria in different biochemical tests media

Name of the test	Reaction	Indication sign	Remarks
Gram staining	-ve	Small, rod shaped, pinkish in colour	Gram staining showed gram negative bacteria
Motility	+ve	Growth area extending away from the inoculation line	Gram negative bacteria showed motile extending growth area formation.
Simmon's citrate test	-ve	Colour did not change from green to blue.	Does not have ability to metabolize Citrate as side carbon source.
Methyl Red test	+ve	Colour turns from yellow to red ring	Bacteria has the ability to ferment glucose.
Catalase test	-ve	There were no oxygen bubbles.	Isolated bacteria did not form bubbles resulting from production of O_2 gas.
Oxidase test	-ve	The colour of oxidase disc did not change to purple.	Isolated bacteria did not change the colour of Oxidase disc to purple.
Triple sugar Iron test	+ve	Colour changed from red to yellow	Bacteria produced H2S at the butt conforming Lactose fermentation.
Urease test	-ve	Colour did not change from yellow to pink	Bacteria does not have ability to break down urea.
VP test	-ve	Colour does not change from yellow to red ring formation on surface.	Bacteria does not have ability to produce acetyl methyl carbinol from glucose fermentation.
Nitrate Reductase test	-ve	Development of red colour after adding Zn dust.	Bacteria does not have ability to produce nitrate reductase enzyme.
Indole test	+ve	Colour changes from yellow to red ring on the surface.	Isolated bacteria have the ability to make Tryptophan enzyme.
Phosphate solubilization test	-ve	No halo zone was observed in the incubation plate.	Bacteria does not show phosphate solubilization activity.

3.3 Antibiotic Sensitivity test

In sensitivity test (Fig P), Ofloxacin, revealed the highest significant antibacterial activity with showing a inhibitory zone. Antibacterial activity study suggested that rain water (Bacterial source), have a broad spectrum of intermediate antimicrobial activity against isolated bacteria with prominent inhibition zone. The above tests provided detail information about the isolation, characterization, and antibiotic sensitivity assay against *E. coli.* The present investigation would be good source of information for molecular detection of this devastating bacteria and design a suitable control technique.

In the present study, Ofloxacin (OF) performed highest antibacterial activity against isolated bacterial with inhibition zone susceptible. This study confirms us the competency of some antibiotic and soil bacteria as natural antimicrobials and suggests the possibility of employing them in drugs for management of infectious diseases caused by *E. coli.*

In the present investigation, the isolated bacteria were gram negative, pink colour, rod shaped and Motile, Triple Sugar Iron agar test, Methyl Red, VP test are positive, whereas Indole Test, Nitrate Reductase Test, Catalase test, Simmon's Citrate test, Oxidase test, Urease test and Phosphate solubilization test are negative.

4. Discussion

The isolation, identification and characterization of microorganisms, useful as bio-control agents or producers of bioactive compounds, are great relevance for the modern and compatible agriculture. In the present research project, the morphological, physiological and biochemical characterization were conducted for bacterial identification. Identification of bacteria five *E. coli* strains were isolated from road side rain water outside the premises of Modi Institute of Science and Technology, Kota, Rajasthan.

In present study, analysis of road side rain water samples from different localities of Kota, Rajasthan, India, indicated

that *E. Coli* is present in this water. After identification, strains that showed close resemblance with *E. coli* were selected to screen for further biochemical analysis. To the best of our knowledge, we are first time reporting the *E. coli* as plant growth promoting rhizobacteria. The presence of growth promoting traits signifies the potential of E. coli to be used as crop inoculants., 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 natural environmental conditions.

Similarly, under natural conditions, bacterial inoculations significantly stimulated vegetative and yield parameters. The colonization of rhizosphere of agronomically important crops by opportunistic human pathogens has been reported by different workers.

Moreover, in previous studies, these bacteria have also been showing to exhibit different plant growth promoting traits including IAA. 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 different 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. *In vitro* screening revealed that strains of waterborne E. coli were showing plant growth promoting traits especially auxin production (Nisma Farooq *et al*.,2014) (For instance, quantity of auxin ranges from 58 to 135 µg ml-I in L-tryptophan amended Auxin production by different strains of *E. coli* in the presence and absence of L-tryptophan. This concentration of auxin was comparable or even higher than rhizobacteria that we previously isolated from natural plant settings (Ali. B *et al*., (2009a) [3], Ali.B *et al*., (2009b) [3], Sadiq. A *et al*., (2013). 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. Moreover, in previous studies, these bacteria have also been known to exhibit different plant growth promoting traits including IAA (Egamberdieva *et al*., (2008), Ali.B *et al*., (2009a) [3], Noreen S *et al*., (2012) [28].

It is experimentally proved that PGPR have positive effect on the growth of different crops and plants (Wu S.C. *et al*., 2005). The impact of rhizobacteria generally on plant growth and health may be classified as neutral, deleterious or beneficial (KloepperJ.W. *et al*., 1989). (Chanway C.P.*et al*., 1989; Glick B.R. *et al*., 1997; ZhangF.*et al*., 1997; BentE.*et al*., 2001) record the ability of microbial inoculants, to increase plant growth and germination rate, improve seedling emergence, responses to external stress factors and protect plants from diseases. The earlier work revealed that use of PGPRs improves seed germination, seedling emergence, seedling vigor and seedling stand over the control (Dipandita *et al*.,2015).Similar results have been reported in other crops such as potato, radish plants, sorghum and pearl millet (Burr T.J.*et al*., 1978;Raju N.S. *et al*., 1999; Niranjan S.R. *et al*., 2004) [26].The improvement in seed germination by PGPR was also found in work with wheat and sunflower(ShaukatK.*et al*.,2006) [37], where it was found that some PGPR induced increases in the seed emergence, in some cases achieving increases upto100% greater than controls. Our results also show the higher seedling with isolated bacterial strains. In pot experiment, it was observed that PGPR inoculation significantly increase the growth of seedlings of Chickpea. In general, inoculation resulted in early seedling growth and development. Similar findings were reported by Dobbelaere S.*et al*., (2006) who assessed the inoculation effect of PGPR *Azospirillum brasilense* on growth of spring wheat. They, observed that inoculated plants resulted in better germination, early development and flowering and also increase in dry weight of both the root system and the upper plant parts (Gravel V.*et al*., 2007) [15]. Soil condition also influenced the growth promotion by bacterial strains. Martinez-Toledo M.V.*et al*., (1988) showed that the numbers of Azotobacterial decreased as plant growth continued in non-sterile agricultural soil, while the numbers of Azotobacterial associated with maize roots grown in sterile agricultural soils remained similar to those of the original inoculum. This may imply rhizobacteria had a more competitive ability to survive and affect the growth of inoculated plants in the presence of indigenous microflora (Khalid A *et al*.,2004) [21]. In this study, Inoculation of PGPR strains increased all parameters determined in-pot experiment as well as in field. Again, Mixed Inoculants which indicate that mixed inoculants. The present experiment revealed that seed inoculation with all isolated bacteria resulted in an increased plant height and leaf numbers. Similar increases in plant height and leaf area were observed in different t crops such as potato, radish plants, sorghum and pearlmillet inoculated with Pseudomonas, Azospirillum and Azotobacterstrains (Burr T.J. *et al*., 1978; Raju N.S. *et al*., 1999 [36]; Niranjan S.R. *et al*., 2004) [26].It was found that three Plant growth promoting rhizobactera (*viz*.EscherichiacoliDACG2, Pseudomonas fluorescence DACG3, Burkholderiasp.DACG1) isolated from the rhizosphere of *Sesbania bispinosa* significantly enhanced root and shoot length and biomass production of chickpea (Dipanvita *et al*.,2015).Therefore it is suggested that the use of PGPR isolates of as effective biofertilizers might be beneficial for cultivation of cereal crops.

Potential of strains as biofertilizers is evident from growth stimulation of V. radiata under axenic and natural environment (Farooq *et al*., 2014). Hence, after biosafety screening, waterborne *E. coli* has a good prospect to be used as plant growth promoting rhizobacteria.

Based on studies done in the past few decades, the presence of environmental *Escherichia* is now well recognized**.** This environmental ***E. coli*** may be of animal-origin and have become adapted to their surrounding environments; or may retain the characteristics of their ancestral linage, which was environmental bacteria using soil and sediment as their primary habitat**.** More data are needed, especially genomic information of environmental *Escherichia* strains, to clarify the evolutionary history of environmental ***E. coli.*** There are many questions still remaining, including***: (i)*** how do these bacteria influence current water quality monitoring? and ***(***ii***)*** are these bacteria potentially pathogenic to humans? ***(***iii***)*** can Escherichia strains be used as PGPRs? Further research is needed to answer these questions**.**

5. Conclusion

Based on research finding the following conclusion have been drawn**.** All water sources were positive for the presence of ***E. coli.*** colonies were mostly available in water which drawn from road side rain water**.** The high counts of indicator (***E. coli)*** in water samples of the study area suggested that the

presence of pathogenic organisms. *E. coli* isolation and identification methodology has been reported in the literature and the isolated and identified *E. coli* was preserved in biology laboratory. The base line information generated from this study may attract other researcher for further studies on *E. coli.*

Based on studies done in the past few decades, the presence of environmental *Escherichia* is now well recognized. This environmental *E. coli* may be of animal-origin and have become adapted to their surrounding environments; or may retain the characteristics of their ancestral linage, which was environmental bacteria using soil and sediment as their primary habitat. More data are needed, especially genomic information of environmental *Escherichia* strains, to clarify the evolutionary history of environmental *E. coli.* There are many questions still remaining, including*: (*i*)* how do these bacteria influence current water quality monitoring? and *(*ii*)* Can these bacteria play potential role in combating abiotic stress and can be used as biofertilizers.

Now days there are growing demands for biologically based agricultural practices. Recent surveys of both conventional and organic growers indicated an interest in using biofertilizer products suggesting that the market potential of biofertilizer products will increase in the coming years. S. marcescens NBRI1213 can therefore be used as a PGPR agent because its application resulted in better growth even under stressed environment. This strain may be helpful in minimizing the impact of temperature stresses which is currently limiting crop production under low input conditions and give rise to a more sustainable agriculture.

Use of Biofertilizers were initiated 100 years back but several constrains were involved in their commercial production like contamination, poor soil quality, combination to inoculated strain, abiotic stress and lack of safety standards. The combination of inoculated strains plays great role in fight with Drought stress, Salinity stress. *E. coli* can be perfectly formulated with PGPRs for the betterment and improvement for desired crop. In future, some other technologies such as next generation sequencing, gene editing are powerful tools to enhance the knowledge of *E. coli* and its application as biofertilizers.

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