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Short Communications

Occurrence of *Proteus mirabilis* Associated with Vegetable Samples in Dehradun, Uttarakhand, India

Heena Shoket[1], S.Ali[1], R.K. Gupta[1] and V.K. Mishra[2]*

[1]Department of Biotechnology, Doon (P.G.) College of Agriculture Science, Dehradun India
[2]Department of Biotechnology, Doon (P.G.) Paramedical College, Dehradun-248001, Uttarakhand, India
**Corresponding author*

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A B S T R A C T

Contamination fruits and vegetables with bacterial pathogens during pre-harvest/post-harvest production system is a serious health concern. Non-composted or improperly composted manure contaminate fruits and vegetables through used a fertilizer/ or in soil amendment/ or in irrigation water. A study on occurrence of human pathogens in spinach and tomato detected the presence of *Proteus mirabilis*. The isolates, *Proteus* sp. SP14 (from spinach) and *Proteus sp*. T3 (from tomato) showed 99% resemblance with *Proteus mirabilis* in nblast analysis. RFLP analysis of PCR amplified 16S rDNA has shown that difference exists in *Proteus mirabilis* strains.

Introduction

Food-borne diseases are a serious public health problem worldwide[1]. During the last few decades, there have been increased incidences of outbreak of diseases due to consumption of fresh vegetables and fruits contaminated with human pathogens[2]. Among the greatest concerns are enteric pathogens i.e., *E. coli*O157:H7, *Salmonella and Proteus spp.* that have the potential for growth even at low infectious doses. Recently, prevalence of *Staphylococcus aureus* , *Bacillus, Proteus* and Yeast bacteria food poisons have been reported in vegetable salads[3]. The fecal contamination of vegetables by microorganisms like *P. mirabilis* is a potential human health risk most commonly causing urinary tract infection (UTI) and infection-related kidney stones[5]. The objective o f this study was to investigate occurrence of *Proteus spp*. in spinach and tomatoes collected from an agricultural field in Dehradun, Uttarakhand, India, and to verify its sensitivity to antibiotics.

From March 2012 to August 2012, Spinach and Tomato fruits were sampled from two different local vegetables growing areas of Dehradun. 25 gm each sample were homogenized in a blender with 225 mL of 0.1% tryptone water for 2 min. 10-15 ml of each homogenate was incubated in modified *tryptone*-Soy *Broth* (mTSB) at 37^0C for 16-20 h (pre-enrichment phase). One milliliter

of the pre-enrichment broth was then sub cultured in 9 mL of Luria–Bertani (LB) broth and incubated at 37^0C for 24 h. 0.1 mL of culture was then placed on MacConkey (HIMEDIA) agar media and blood agar base plate, supplemented with 10% sheep blood. The isolates, SP14 from spinach and T3 from tomato were characterized by various standard biochemical tests [Table 1], which indicated suspected *Proteus spp*. Furthermore characterization of the isolates (SP14 and T3) were performed by 16S rRNA gene sequence analysis.

Genomic DNA was isolated from each strain using a fast DNA kit (Q-Biogene). Partial 16S rRNA gene sequence was amplified by polymerase chain reaction (PCR) using primer sets 16sF (5´-GAGTTTGATCCTG GCTCAG-3´) and 16sR (5´GGTTACCTT GTTACGACTT-3). 50 microlitre reaction mixture 100 ng of total DNA, 2 U of Taq polymerase, 0.2mM of dNTPs 3.0 mM of $MgCl_2$ and 0.4 μM of each primer. The PCR amplifications were carried out using initial denaturation step of 2 min at 94^0C, followed by 30 cycles 1 min 94^0C, 30 s 55^0C, 72^0C for 1 min. The reaction was completed at final extension temperature 72^0C for 10 min. The presence of PCR products was determined by electrophoresis of 10 μL of the reaction product in a 0.8 % agarose gel. The amplicon size was 1.5 kb [Figure 1a]. Purified PCR products were sequenced using ABI BigDye Terminator chemistry v3.0 (Applied Biosystems). Partial 16 s rDNA sequences of strain SP14 and T3 were deposited in Gene Bank and assigned NCBI accession no. JX576494 (*Proteus sp.* SP14) and JX576495 (*Proteus sp.* T3). Partial 16S rDNA sequences obtained was compared directly with sequences in the NCBI database using nBLAST. The partial 16S rDNA sequences of the isolated strains, SP 14 and T3 were highly similar (99%) to *P. mirabilis* (Fig. 2). PCR product of each strain was digested with restriction enzyme Hae 1 following manufacture protocol for 16 h at 37^0C. PCR-RFLP pattern revealed differences in *Proteus sp.* SP14 (strain SP14) and *Proteus sp.* T3 (strain T3) [Figure 1b].

Antibiotic resistant pattern of *Proteus mirabilis* strains, SP14 and T3 were tested for susceptibility to 13 antibiotics on Möeller-Hinton Agar medium using the Clinical and Laboratory Standards Institute (CLSI) guidelines[6].

Figure.1.a PCR amplification of 16S rDNA of bacterial strain *Protus sp.* T3 and *Proteus sp.* SP14 (from tomato and spinach, respectively); Fig.1b. Restriction digestion of 16S rDNA of T3 and SP14 strains with RE Hae 1

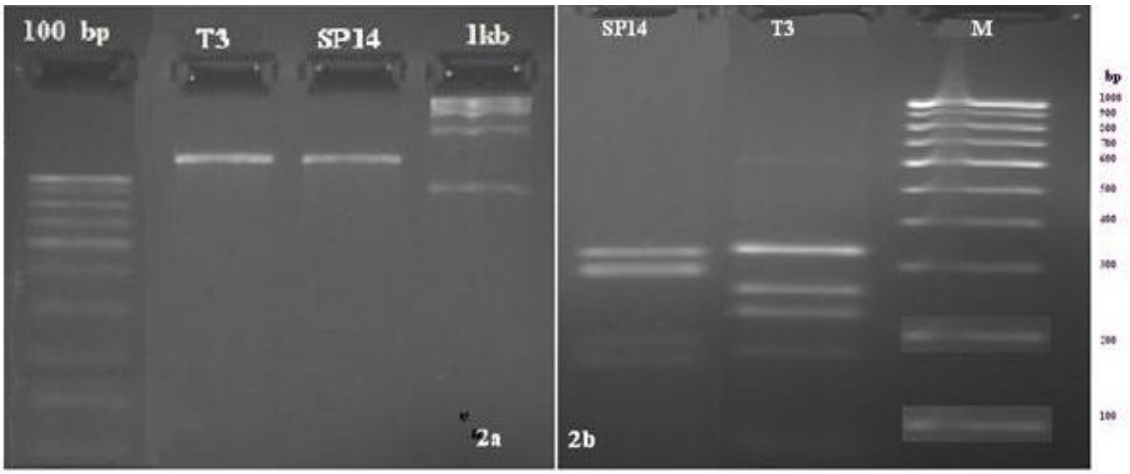

Table.1 Biochemical test of Bacterial isolates, SP14 from spinach and T3 isolate from tomato

Test	Bacterial Strains	
	SP14 (Spinach)	T3 (Tomato)
Indole production	-	-
Methyl red	+	+
Voges-Proskauer	-	+
Hydrogen sulfide (on TSI agar)	+	+
Ornithine decarboxylase	+	+
Lactose	–	-
D-Glucose	+ (Acid & Gas production	+ (Acid & Gas production
D-Sorbitol	-	-
d-Xylose	+	+
CT-SMAC	-	-

Figure.2 Phylogenetic analysis of isolates, SP14 from spinach (NCBI accession no JX576494, *Proteus sp.* SP14) and T3 (accession no. JX576495, *Proteus sp.* T3)

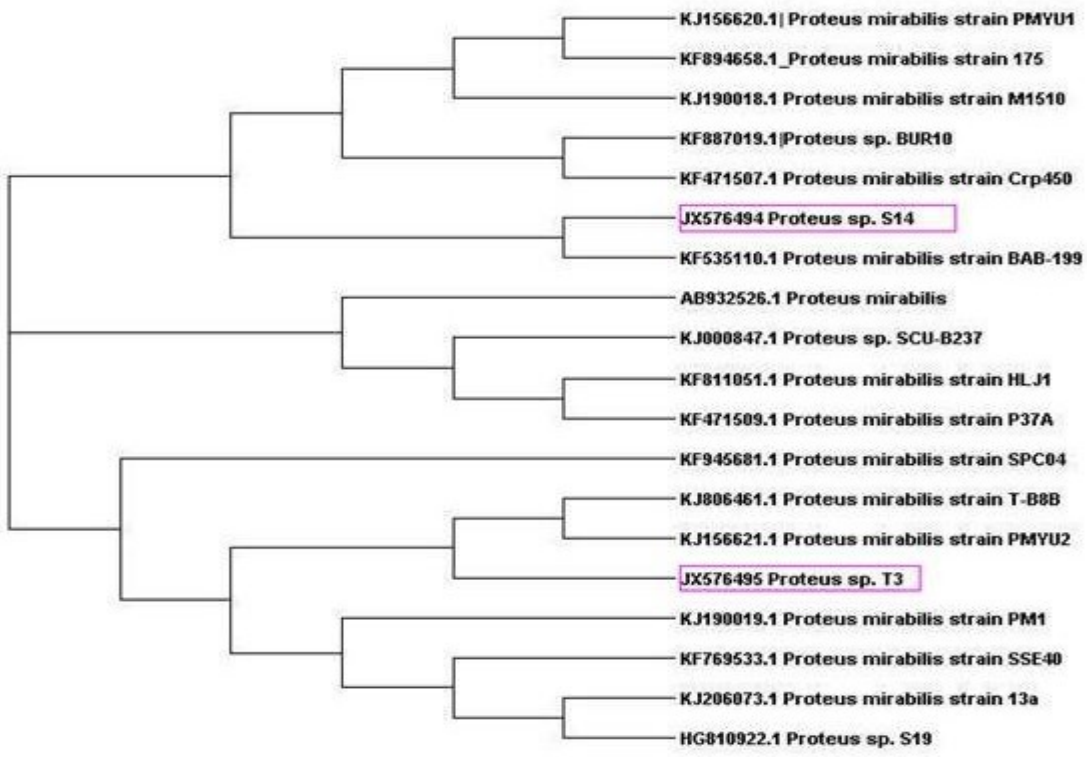

Table.2 Antibiotic resistance of T3 isolate from tomato (*Proteus sp*. T3) and SP14 from spinach *(Proteus sp.* SP14) (Concentrations of antibiotics are given per mL)

S.no.	*Antibiotics*	Bacterial Isolates	
		Proteus sp. T3 (Tomato)	*Proteus sp. SP14* (Spinach)
1	Penicillin-G (2 U)	R*	R
2	Chloramphenicol (30 μg)	SR	SR
3	Erythromycin (30 μg)	R	S
4	Tetracycline (30 μg)	S	S
5	Ampicillin (10 μg)	R	R
6	Vancomycin (30 μg)	S	R
7	Amoxyclav (30 μg)	R	SR
8	Gentamicin (30 μg)	S	S
9	Ofloxacin (30 μg)	S	S
10	Cefoxitin (30 μg)	R	R
11	Streptomycin (30 μg)	S	S
12	Sulfametoxazol-trimethoprim (30 μg)	R	R
13	Lomefloxacina (10 μg)	S	S

* R, resistant (< 5 mm); SR, Semi Resistant (5-12 mm); S, sensitive (> 12 mm)

Analyses of resistance to antibiotics in the SP14 and T3 used for the present study showed resistance to penicillins G, ampicillin , and cephalosporin (cefoxitin), amoxyclav, and Sulfametoxazol-trimethoprim while being sensitive to Tetracycline, Gentamicin, Ofloxacin and Lomefloxacina [Table.2]. Occurrence of *Proteus mirabilis* antibiotic resistant strain in tomato and spinach will pose serious environmental and health risk.

Acknowledgements

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