INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 17, No. 2, p. 207-217, 2020

RESEARCH PAPER OPEN ACCESS

Molecular identification of Bacteria causing soft rot disease of tomato in Mansehra, Pakistan

Naseeb Ullah*[1], Aziz Ud-Din[1], Tariq Mahmood[1], Hamid Ali[1], Wajid Ali[1], Sajidul Ghafoor[1], Shakir Khan[1], Safa Faqir[1], Raja Asad Ali Khan[2]

[1]*Department of Genetics, Hazara University, Mansehra, Pakistan*

[2]*Department of Plant Pathology, Agriculture University, Peshawar, Pakistan*

Key words: Tomato (*Lycopersicum* sp.), Bacterial pathogens, 16S rRNA, Sequencing, Polymerase chain reaction (PCR)

http://dx.doi.org/10.12692/ijb/17.2.207-217 Article published on August 30, 2020

Abstract

Vegetables are regarded as the prolonged plant parts which are used either cooked or uncooked. Different vegetables have different nutritional values providing vitamins, antioxidant, minerals, fibers for the normal functioning of the human body. Pakistan is an agriculture country and above 70% of its population depends on agriculture. Mansehra is the North-West district of the province Khyber Pakhtunkhwa, Pakistan. It is famous for tomato (*Lycopersicum esculentum*) cultivation, However, there are certain limiting factors which lower the yield of tomato. Besides others the most important yield limiting factor is diseases caused by bacteria. These diseases pose a serious threat to the quality and quantity of tomato. We collected symptomatic samples of tomato showing symptoms of soft rot in the year 2016-2017. Bacteria were isolated and were further cultured to get pure colonies. We applied biochemical tests and molecular markers for the identification of isolated bacteria. The isolated bacteria were further subjected to PCR and we sequenced partially their *16S rRNA*. It was revealed that Erwinia and pseudomonas were the causing agents. The obtained sequences of *16S rRNA* were submitted to NCBI gene bank having accession numbers MH244345.1 and MH244346.1

* **Corresponding Author:** Naseeb Ullah ✉ naseebullah43@yahoo.com

Introduction

Vegetables are the prolonged part or shoots of plants that are used as a food. These are then used in different ways either cooked or uncooked (Yusuf *et al.*, 2004). Vegetables are important for providing the vigor and immunity to the body. They provide vitamins and other essential nutrients needed for healthy life and proper functions.

Tomato is grown, consumed and are placed at 6th among 15 vegetables which are consumed worldwide. Tomato and onion are used in a variety of ways including soup, sauce and other cooking factories (Lohano and Mari, 2005). It is considered beneficial and cash crop which can be grown in open fields as well as in green houses (Ali *et al.*, 1994). Tomato are grown throughout the Pakistan on an area of 63200 ha which produce 599700 tonnes in which the contribution of Khyber Pakhtunkhwa province is prominent producing 135700 tonnes by cultivating on in area of 1400 ha. In District Mansehra it is grown as a kharif crop which is from June to September (PAKISTAN, 2015-2016). Tomato is eaten either cooked or uncooked. It is rich source of Antioxidant, Vitamin A and also contains minerals and fibers that are essential for health. Number of genes are present in different variants controlling different protein of lycopene and cyanine needed as an antioxidant for human body (Stahl *et al.*, 2001).

Soft rot which are caused by bacterial pathogen i.e. *Erwinia* sp. which is one of the crucial diseases of tomato crop resulting the decrease in yield and lower the value of tomato throughout the world (Agrios, 1997; Farrar *et al.*, 2000; Van der Wolf *et al.*, 2017; Ozturk *et al.*, 2018). *Erwinia* sp. is one of the gram-negative bacteria, which is rod shaped, anaerobic, motile with flagella oriented at all side are soil born and tuber transmitted. It is the causal agent of devastation of tomato and other economic crops at both the fields and at the store houses. The *Erwinia* genus constitutes *carotovora* subsp. *atroseptica*, *carotovorum* subsp. *brasiliensis*, *chrysanthami*, *Pectobacterium* (*Erwinia*) *wasabiae*. In these species E. *carotovora* and E. *atroseptica* are the prominent in the destruction of tomato (Pitman *et al.*, 2010). *Chrysanthami* sp. of *Erwinia* can tolerate in both cold and warm climatic conditions (Hannukkala and Segerstedt, 2004).

Tomato is chief substrate for various bacterial pathogens. These pathogens are either soil born or mainly seed born. Therefore, for healthy and disease-free tomato crop the proper check and balance is mandatory. For this purpose, the testing of seeds by ordinary technique is slightly difficult and not so accurate. However, another important technique for pathogen identification which is more accurate and reliable is the use of PCR in which the gene specific markers are used for different bacterial and other diseases. Here in this study we have collected symptomatic samples of tomato showing signs of bacterial diseases especially soft rot. we further identify the causing bacteria of soft rot through molecular approaches.

Tomatos are important for health and growth of human being and provide vitamins and immunity to the body. Tomato is grown on greater area in Mansehra, Pakistan but the production is lesser due to loss mainly due to bacteria. We identify *Erwinia* and *Pseudomonas* bacteria as a causal agent for the destruction of tomato in tomato. As these bacteria flourish well in humid and lower temperature. Due to these conditions these bacteria lower the quality and quantity of tomato production.

So, the current project was designed to confront the future challenges of tomato production in Pakistan especially in Mansehra against the virulent races of Bacteria. These bacteria cause great loss to the tomato crop. Therefore, this project will be efficient for the improvement of the tomato crop production in Mansehra, Pakistan.

Materials and methods

Collection of Pathogen from Tomato

Tomato (*Lycopersicum esculentum*) fields at Mansehra, Khyber Pakhtunkhwa, Pakistan were visited and symptomatic samples were collected made from them (Fig. 4). Moreover, the symptomatic samples were collected including leaf, stem and fruit

in the paper envelop by detaching it with sterilized razor and scissor. The paper envelopes was labelled and were kept for the safe transport of pathogen to the Molecular Genetics laboratory, Department of Genetics Hazara University, Mansehra, Pakistan.

Bacterial isolation

The leaves and fruits of tomato were cut into small pieces and then surface sterilized and washed with pure water. After that, grinded in 0.84% in NaCl solution. The suspension was then put in test tube containing 1ml pure distilled water. Then from the test tube the streaking of bacteria was done. After streaking the petri plates were inverted and placed for 36 to 48hrs at 28-30°C. Different colonies arise on nutrient agar including transparent, whitish creamy color, and non-flat. Colonies selected were transferred to another fresh medium plate. This was repeated several times for obtaining the pure culture of bacteria (Fig. 5).

Nutrient is non-specific medium means every type of microorganism tends to grow including saprophytes and un related bacteria and fungus. First for the fungus Fungone® containing Fluconazole were applied. After the culture the pure colonies were selected and picked for further studies by toothpick and were store in glycerol stock of 75% and were kept at -20°C. For re-culturing the bacteria were streaked on the nutrient agar medium and kept at 28°C for 2 days.

DNA Extraction

For the extraction of DNA, the bacterial isolated were cultured overnight at 28°C in 5ml LB broth medium in chamber. Then from every isolates 1.5ml was taken in Eppendorf tube and centrifuged at 8000 rpm for 10 min, supernatant was removed and then dissolved in 100µl of Nuclease free sterile water. For ease and efficient result, we choose the water boiling method for the extraction of DNA from bacteria. In this technique bacterial cells are broken down by heat (De Boer and Ward, 1995). We increase the temperature of H_2O up to 96°C for the duration of 6 min with a little change in the previously filed method. In this way, the bacterial culture was purified and then a single was taken into 100µl of ddH_2O then boiled up to 96°C for 6 min then placed directly changed in -20°C which is ready for further use of investigation and were used as 5µl as a template in each PCR reaction.

Biochemical test for the identification of bacteria

Significant amount of infected tomato was accumulated from different areas of District Mansehra during investigation and observation during the field survey. The culture was made in growth chamber at 27±2°C and were observed that the colonies were creamy and shiny, raised and circular type. Different biochemical test were performed for the identification and the resulted product was made comparable with (Lelliott and Dickey, 1984; Perombelon and Kelman, 1980).

Molecular characterization

For the precise characterization of bacteria was performed by applied biosystems 2720 Thermal cycler of life technologies. For using PCR the specific primer for Eca was designed as Eca1F (CGGCATCATAAAAACACG) Eca2R (GCACACTTCAT CCAGCGA) according to (De Boer and Ward, 1995) having amplification up to 690bp. Whereas, 16S universal primer as Forward 9F (GAGTTTGATCCT GGCTCAG) and 1510R as (GGCTACCTTGTTACGA). For PCR enzynomics kit was used 20µl reaction was made in such way that it contains the following ratio (Table 3) and (Fig. 3).

Gel Electrophoresis

The PCR product was then visualized by 1-1.5% agarose Gel. Agarose was dissolved in TAE buffer for heating in oven for 3min and then place at room temperature for 50 min add 8µl EtBr and then poured in to medium gel tray in which comb for wells was fitted. Then 7µl of PCR product was mixed with loading dye and then wells were loaded run at 80volts for 55min along with the 1kb ladder. Then for the gel image VU light was used under Gel DOC (Biodigonstic). This process was handled with care and prevention because EtBr and VU light are highly carcinogenic.

Gene purification

After using universal primer of *16S rRNA* at the applied biosystems 2720 Thermal cycler of life

technologies run on 1% agarose for 55 min and then cut the visualized bands under UV light (Fig. 7) for gene cleaning and transfer it to 1.5ml Eppendorf tube. For gene purification SanPrep column DNA Gel Extraction Kit (Sangon Biotech) was used.

Sequencing

After using *16S rRNA* at automated thermos cycler the amplified product was purified and 15µl was sent to MACROGEN, Korea for sequencing analysis. The result given by MACROGEN were analyzed by different ways for investigation.

Results and discussion

For the pathogenicity of pathogen different fields at different times were visited. They differ at different fields and some tomato crops were greatly infected and was hardly to purify its bacterial culture. The tomato was greatly infected at different location, but pathogenicity was in abundance in the Marghazar, Battal and Jaborri. It mainly depends upon the temperature so, the hilly areas along with high altitude the temperature falls down which favors greatly the *Erwinia carotovora sub* sp. *atroseptica* resulting black leg and soft rot in tomato as filed earlier by (Pérombelon and Kelman, 1979). In Mansehra district more tomato is cultivated in Marghazar and Ghandia area. The Shinkiari and Ghandia is at lower altitude and the temperature is relatively high than other elevated areas but the pathogens attack were greater than our plotted results as result was found by (Oliveira *et al*., 2003). Here it is also possible that soft rot may not be due to *Eca* but the causing agent here is may be *Ecc* which is recorded destructive and likes higher temperature is already filed by (Oliveira *et al*., 2003). This argument was supported by our research work when we investigate the collection from low elevation having relatively higher temperature were characterizing as *Ecc* strain.

At this present research work we also recorded the presence of *Enterobacter sp*., *Streptomyces* species at the fields where we were collecting. This was present in humid condition in the tomato as described by (Iftikhar *et al*., 1993).

PCR of Pathogens infecting vegetables

Some characteristic of the pathogens comes in similarity with each other in such characteristics which are used for the identification and differentiation. For this propose we analyzed these pathogens with help of PCR which is precise than the others way of identification. The *Eca* specific primer gives amplification of 690bp as filed by (De Boer and Ward, 1995) which amplified *Eca* strain (Fig. 6). This technique is efficient and regarded accurate for identification of *Erwinia* strains. In this method the Taq polymerase enzyme plays important role in amplification of different bands. Second, the procedure of the PCR plays a milestone role in the identification process. The PCR may be simple or Multiplex, in simple only one primer is used only for single strain if there are more bacterial strain are expected than multiplex PCR is efficient using different primers at a same time.

Biochemical test for the identification of bacteria

Different biochemical test was performed for the identification. After deriving results some isolated strains did not produce acid from α-methyl glucoside and reducing substance from sucrose as the all produces which is sign of that without these all other were gram -ve *Eca* qualities as investigated by (Lelliott and Dickey, 1984). some isolates were able to grow at 36°C which is not the characteristic of *Eca* but refers to *Ecc* and *Ech*. The pseudomonas requires 28°C (Fig. 10) for their culture and our results showed that some *Eca* strain also grow at 36°C (Fig. 11). based on the others investigators consequences that *Eca* show also the characteristics like that of *Ecc* and *Ech* (Thomson *et al*., 1981) and moreover, it is filed by (El-Hendawy *et al*., 2002) that their 21 different *Eca* were found to be able to grow at 36°C while it was filed from previous work of (Jabuonski *et al*., 1986) that *Ecc* which favors high temperature not grown at 37°C. According to erythromycin sensitivity, our isolates were not surprised in major number while some showed the sensitivity and some were less sensitive. According to previous work, *Eca* and *Ecc* may be sensitive and either unsensitive to antibiotics (Lelliott and Dickey, 1984; El-Hendawy *et al*., 2002; Perombelon and Kelman, 1980; Tamang *et al*., 2005).

In our work some species were found that shows medium line i.e. both the characteristic so, for differentiation PCR identification were subjected.

Sequencing

After using 16S rRNA at automated thermocycler the amplified product was purified and 15µl was sent to MACROGEN, Korea for sequencing analysis. The result given by MACROGEN were analyzed by different ways for investigation and were submitted to NCBI Gene Bank and give the accession No MH244345.1 and MH244346.1 showing *Erwinia* and *Pectobacterium sp*. So, these were regarded as the most crucial for the vegetables in Mansehra district.

>MH244345.1

GGCAATGGCGGCAGCTACACATGCAGTCGACGGTA
GCACAGAGAGCTTGCTTCGGGTGACGAGTGGCGGA
CGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAG
GGGGATAACTACTGGAAACGGTAGCTAATACCGCA
TAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGC
CTCTTGCCATCAGATGTGCCCAGATGGGATTAGCTA
GTAGGTGGGGTAACGGCTCACCTAGGCGACGATCC
CTAGCTGGTCTGAGAGGATGACCAGCCACACTGGA
ACTGACACACTGTCCACACTCCTACCGGAGGCAGCA
GTGGGGAATATTGCTCAATGGGCGCAAGCCTGATG
CTGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGT
TGTAAAGTACTTTCACCGGGGAGGAAGGCGAAAAG
GGTAATAACCTTGTCCAGTGTCGTTACCCGCCA

Dendrogram of sample MH244345.1

The Dendrogram of the MH244345.1 as shown in (Fig. 1). representing the comparison of DNA sequence amplified through 16s RNA universal primer to the ten samples most similar downloaded from NCBI Genebank, based on maximum homology. The tree is divided into three clades/groups. According to the tree the sample is more is closely related with the NR_118568.1, NR_159255.1 and NR_118227.1 as shown in (Table 1).

Table 1. Pairwise similarity of MH244345.1 with other bacterial strains.

S.No.	Genus	Species	Strain	Accession No	Pair wise Similarity
1.		*Pectobacterium polaris*	NIBI O1006	NR_159255.1	94%
2.		*Erwinia billingiae*	Billing E63	NR_104932.1	93%
3.		*Enterobacter cloacae strain*	ATCC 13047	NR_118568.1	95%
4.		*Pectobacterium carotovorum*	CFBP2046	NR_118227.1	92%
5.	*Pectobacterium/*	*Erwinia tasmaniensis*	Et1/99	NR_074869.1	93%
6.	*Erwinia*	*Erwinia chrysanthemi*	DSM 4610	NR_117738.2	93%
7.		*Erwinia billingiae*	LMG 2613	NR_119255.1	93%
8.		*Pectobacterium parmentieri*	RNS 08-42-1A	NR_153752.1	93%
9.		*Erwinia billingiae*	LMG 2613	NR_118431.1	93%
10		*Erwinia amylovora*	DSM 30165	NR_041970.1	93%

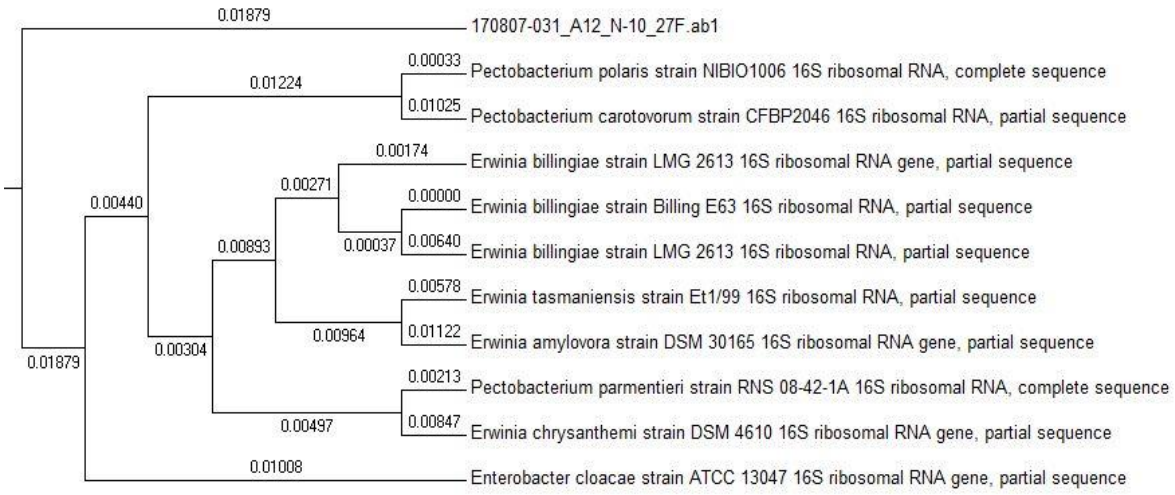

Fig. 1. Phylogenetic tree of sample MH244345.1.

>MH244346.1

CAGCTACACATGCAGTCGAGCGGTAGCACAGGAGA
GCTTGCTCTCGGGTGACGAGCGGCGGACGGGTGAG
TAATGTCTGGGAAACTGCCTGATGGAGGGGGATAA
CTACTGGAAACGGTAGCTAATACCGCATAATGTCG
CAAGACCAAAGTGGGGGACCTTCGGGCCTCATGCC
ATCAGATGTGCCCAGATGGGATTAGCTAGTAGGTG
GGGTAACGGCTCACCTAGGCGACGATCCCTAGCTG
GTCTGAGAGGATGACCAGCCACACTGGAACTGAGA
CACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGG
AATATTGCACAATGGGCGCAAGCCTGATGCAGCCA
TGCCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAA
GCACTTTCAGCGGGGAGGAAGGCGGTGAGGTTAAT
AACCTCGCCGATTGACTTTTTCCGCAAAAAAAGCAC
CCGAGTCCTCTGTGCCTGCAGCCCGCTAATACGGA
GGGAGTAAGCGTTATTCGGAACTACTGGTGCTAAG
CCCCCTCCTTTGTTCTGTCAATTTCCATGTAATCATA
CTGTCATGCCGTGCAACTGCCCTCGAAACTGCCAG
GCTTGAGTCTTTTAATGCGACACAACCTCA

Dendrogram of sample MH244346.1

The Dendrogram of the MH244346.1 as shown in (Fig 2) representing the comparison of DNA sequence amplified through 16s RNA universal primer to the ten samples most similar downloaded from NCBI Genebank, based on maximum homology. The tree is divided into three clades/groups. According to the tree the sample is more is closely related with the NR_026047.1, NR_118293.1and NR_037102.1 as shown in (Table 2).

Table 2. Pairwise similarity of MH244346.1 with other bacterial strains.

S. No.	Genus	Species	Strain	Accession No	Pair wise Similarity
1.	*Pectobacterium/ Erwinia*	*Pectobacterium carotovorum*	212	NR_118228.1	92%
2.		*Pectobacterium atrosepticum*	CFBP 1526	NR_118295.1	92%
3.		*Pectobacterium wasabiae*	SR91	NR_026047.1	92%
4.		*Erwinia chrysanthami*	DSM 4610	NR_117738.2	92%
5.		*Erwinia billingiae*	LMG 2613	NR_118431.1	92%
6.		*Pectobacterium carotovorum*	CFBP2046	NR_118227.1	92%
7.		*Pectobacterium carotovorum*	212	NR_118224.1	92%
8.		*Pectobacterium cacticida*	1-12	NR_037102.1	92%
9.		*Erwinia persicina*	LMG 11254	NR_119364.1	92%
10		*Pectobacterium wasabiae*	CFBP 3304	NR_118293.1	93%

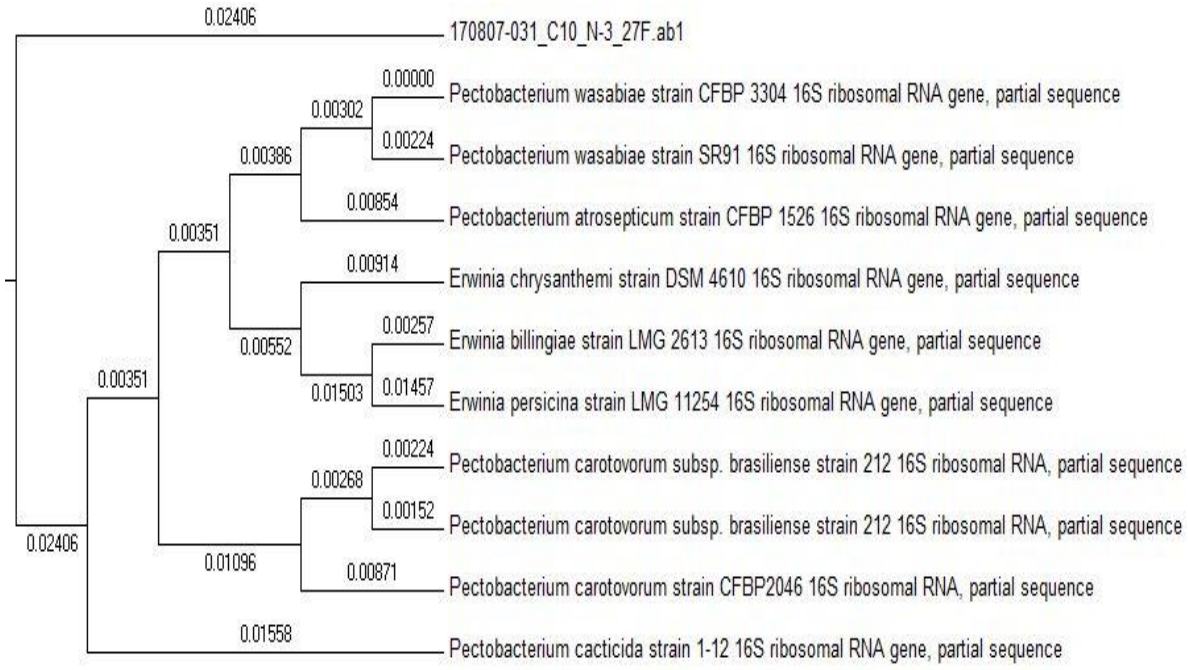

Fig. 2. Phylogenetic tree of sample MH244346.1.

Table 3. List of PCR Master Mix solution and their quantity in PCR used in this study.

S. No	Ingredients	Each reaction (1x)
1	ddH_2O	7.2µl
2	10x buffer (Mg^+ free)	2µl
3	dNTP	2µl
4	$MgCl_2$	1.5µl
5	Primer-F	1µl
6	Primer-R	1µl
7	Taq DNA Polymerase	0.3µl
8	Template	5µl

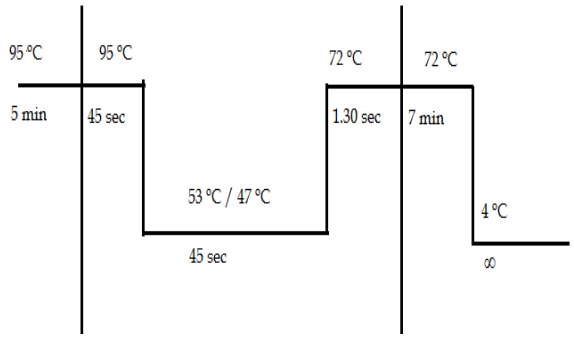

Fig. 3. Graphical representation of PCR profile for 16s RNA and Gene specific primer.

Fig. 4. Tomato fruits showing bacterial infection at different fields in Mansehra, Pakistan.

Fig.5. Representation of pure Bacterial culture isolated from samples of infected tomato from different fields in Mansehra, Pakistan.

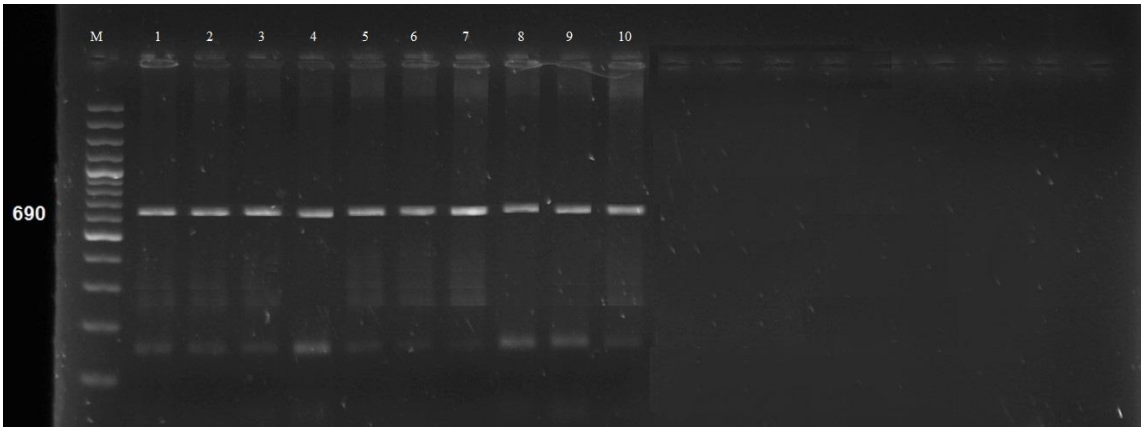

Fig. 6. PCR amplification pattern of *Eca* specific primer. The Eca primer produces characteristic amplicons of 690bp from the soft rot of vegetables. On the left-hand side of the agarose gel (1.5%) M is a 100bp lambda DNA ladder (Fermentas life sciences), while 1,2 are from (Shinkiari), 3 (Baffa), 4,5 (Marghazar), 6,7 (Dhodial), 8 (Shergerh) and 9, 10 from tomato fields of (Battal).

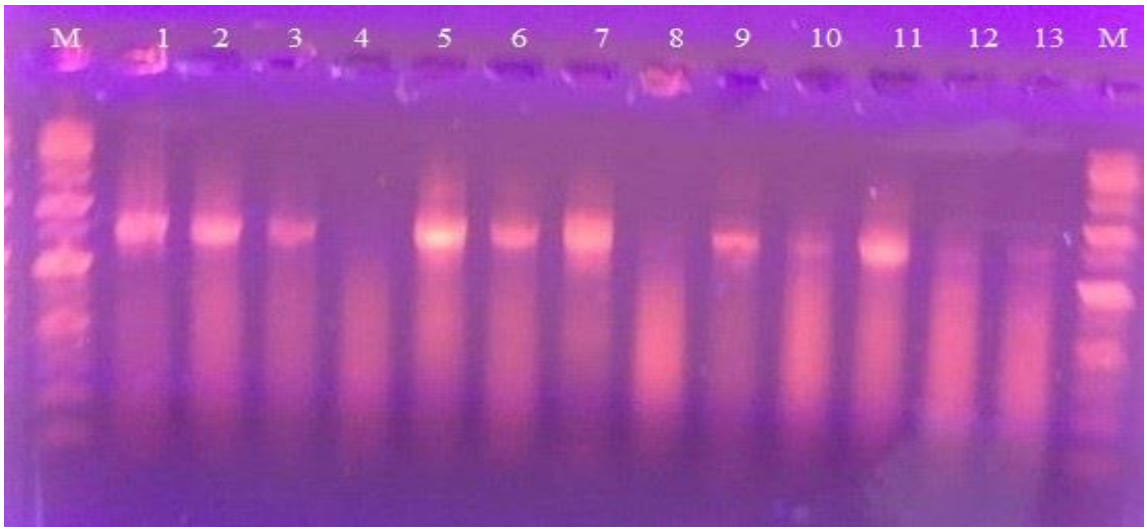

Fig. 7. Amplification with *16S rRNA* universal primer of bacterial DNA samples. On the left-hand side and right hand side of the agarose gel (1.5%) M is a 100bp lambda DNA ladder (Fermentas life sciences).

Fig. 8. Arrow showing the Threads formed by *Erwinia and Pseudomonas sp*. during KOH test, shows that it is gram negative bacteria.

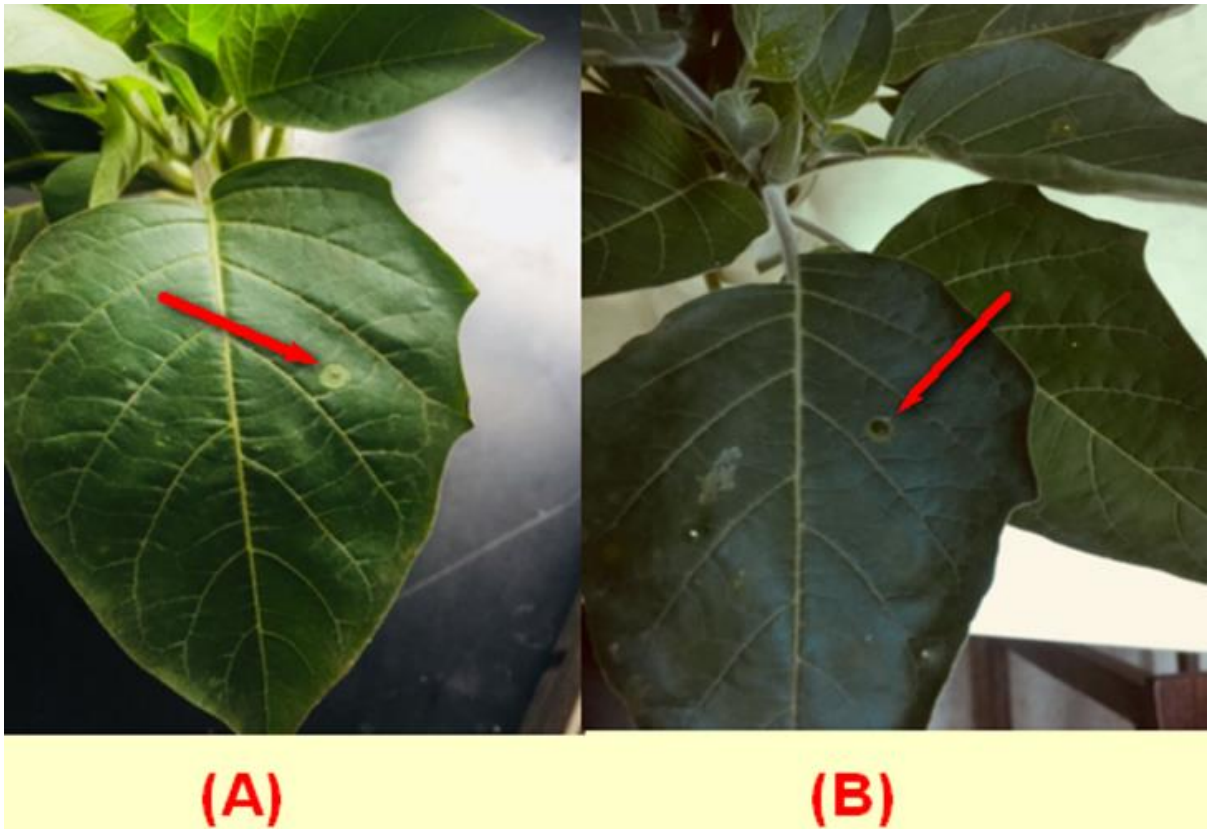

Fig. 9. Effect of Hypersensitive Response test (A) necrotic lesion produced by *Pseudomonas sp*. (B) necrotic lesion created by *Erwinia sp*. on Datura plant leaf.

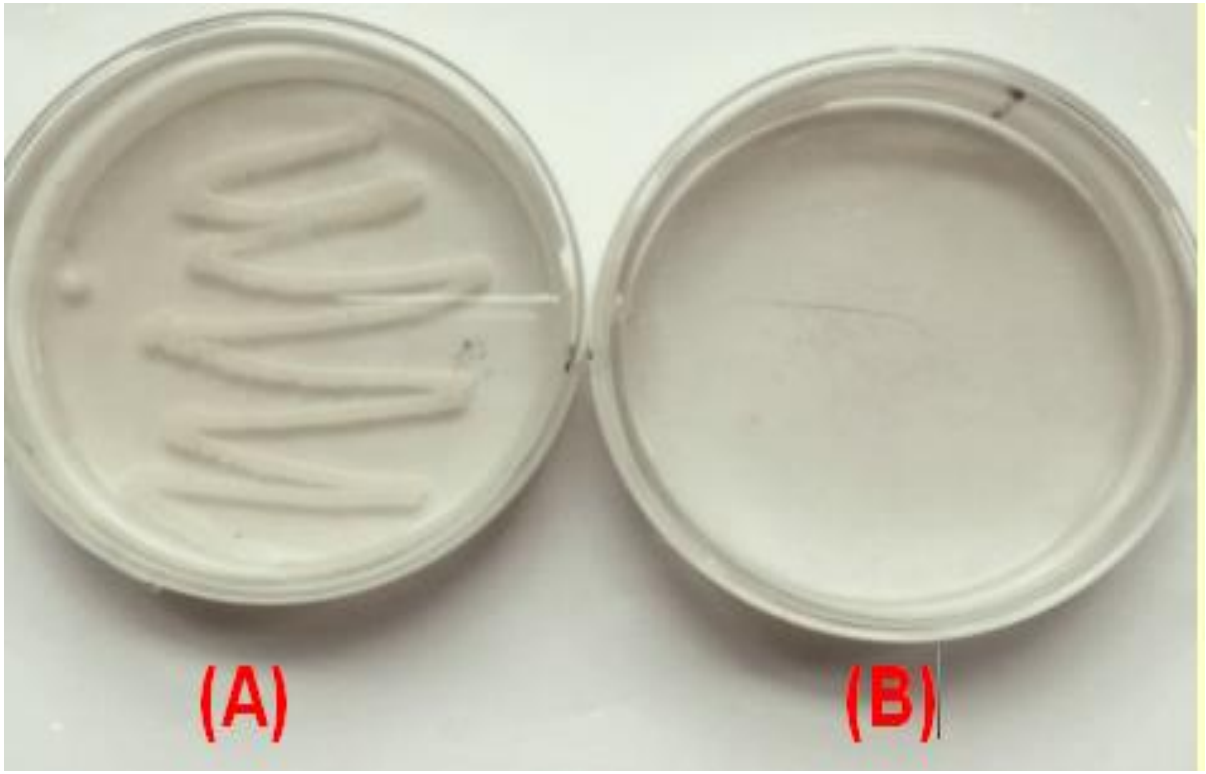

Fig. 10. Effect of temperature on the growth of *Pseudomonas sp*.(A) Growth of the bacteria at 28°C (B) No growth when streaked plate was incubated at above 28°C.

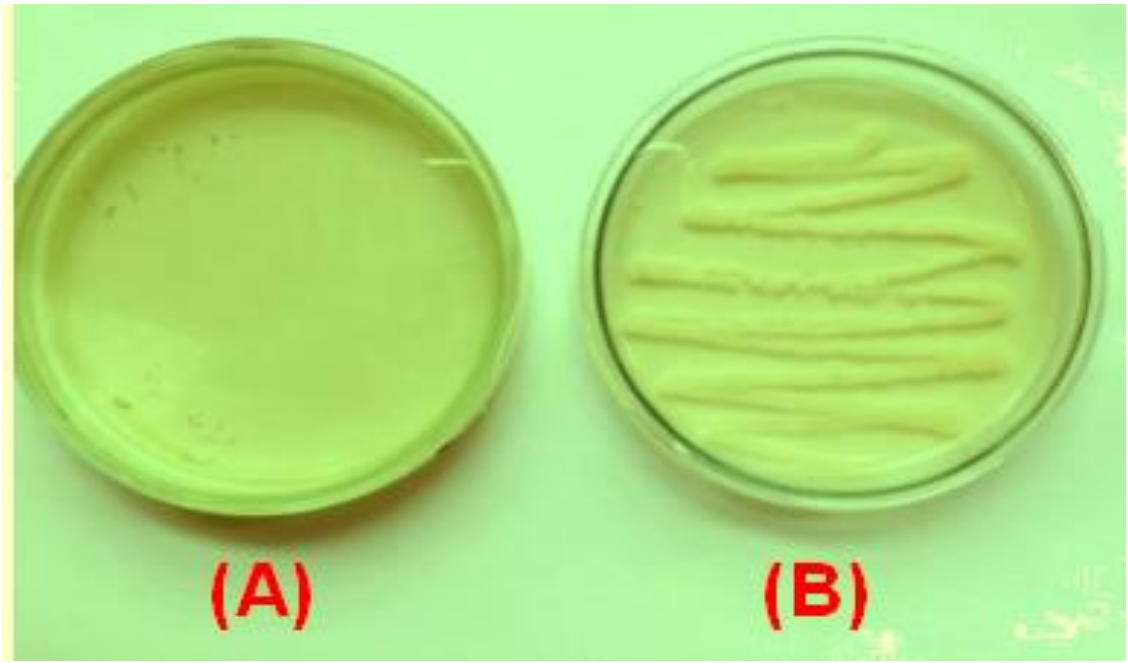

Fig. 11. Effect of temperature on the growth of *Erwinia sp.* (A) No Growth of the bacteria at 36°C. (B) Growth of bacteria when streaked plate was incubated at below 36°C.

Conclusions

in our study it was recorded no specific characterization was set up for identification of the *Erwinia sp.*, so it was concluded that on normal observation characterization of the *Erwinia* is not possible. because most species show divergent from their prescribed characters. so, for authentic characterization the molecular and specific markers, *16S rRNA* identification and Sequencing can enhance our results and precise our characterization.

References

AGRIOS GN. 1997. Plant diseases caused by prokaryotes: bacteria and mollicutes. Plant pathology **4,** 407-470.

Ali F, Parikh A, Shah M. 1994. Measurement of profit efficiency using behavioural and stochastic frontier approaches. Applied Economics **26,** 181-188.

De Boer S, Ward L. 1995. PCR detection of Erwinia carotovora subsp. atroseptica associated with potato tissue. Phytopathology **85,** 854-858.

El-Hendawy H, Osman M, Ramadan H. 2002. Pectic enzymes produced in vitro and in vivo by Erwinia spp. isolated from carrot and pepper in Egypt. Journal of Phytopathology **150,** 431-438.

Farrar J, Nunez J, Davis R. 2000. Influence of soil saturation and temperature on Erwinia chrysanthemi soft rot of carrot. Plant disease **84,** 665-668.

Hannukkala A, Segerstedt M. 2004. Epidemiology, diagnostics and control of blackleg and soft rot on potato Maa-ja elintarviketalous 41. 60 pp.

Iftikhar S, Ahmad I, Soomro M, Aslam M. 1993. Powdery and common scab in spring potato crop in irrigated plains of Central Punjab and Pabbi area of North West Frontier Province (NWFP), Pakistan.

International Commission of Microbiological Specification for Foods. 1998. Microorganisms in Food, Microbial Ecology of Food Commodities. New York Blackie Academic and Professional. Vol **6,** 617-620.

Jabuonski R, Takatsu A, Reifschneider F. 1986. Avaliação da patogenicidade de bactérias do gênero Erwinia isoladas de batateira, tomateiro e de outras plantas hospedeiras. Fitopatologia Brasileira **11,** 587.

Khan AB, Panhwar WA. 2017. Population of Thrips tabaci Lindeman, 1889 in Onion Crop from district Mansehra, Khyber Pakhtunkhwa, Pakistan.

Lelliott R, Dickey S. 1984. Erwinia Winslow *et al.* Bergey's Manual of Systematic Bacteriology (NR Krieg, and JG Holt, eds.) **1,** 469-476.

Lohano HD, Mari FM. 2005. Spatial price linkages in regional onion markets of Pakistan. Journal of Agriculture and Social Sciences **1,** 318-321.

Naz F, Haq IU, Asghar S, Shah AS, Rahman A. 2011. Studies on growth, yield and nutritional composition of different tomato cultivars in Battal valley of district Mansehra, Khyber Pakhtunkhwa, Pakistan. Sarhad J. Agric **27,** 569-571.

Oliveira AM, Duarte V, Silveira JR, Moraes MG. 2003. Incidence of pectolytic erwinias associated with blackleg of potato in Rio Grande do Sul. Fitopatologia Brasileira **28,** 49-53.

Ozturk M, Aksoy HM, Potrykus M, Lojkowska E. 2018. Genotypic and phenotypic variability of Pectobacterium strains causing blackleg and soft rot on potato in Turkey. European journal of plant pathology **152(1),** 143-155.

Pakistan GO. 2015-2016. Ministry of national food security and research (nfs&r).

Pérombelon M, Kelman A. 1979. Population dynamics of Erwinia carotovora and pectolytic Clostridium spp. in relation to decay of potatoes [Erwinia carotovora carotovora, Erwinia carotovora atroseptica]. Phytopathology.

Perombelon MC, Kelman A. 1980. Ecology of the soft rot erwinias. Annual review of phytopathology **18,** 361-387.

Pitman AR, Harrow SA, Visnovsky SB. 2010. Genetic characterisation of Pectobacterium wasabiae causing soft rot disease of potato in New Zealand. European Journal of Plant Pathology **126,** 423-435.

Smeda N. 2009. District profile Mansehra. Small and Medium Enterprises Development Authority, Ministry of Industries and Production. Government of Pakistan.

Stahl W, Heinrich U, Wisema S, Eichler O, Sies H, Tronnier H. 2001. Dietary tomato paste protects against ultraviolet light–induced erythema in humans. The Journal of nutrition **131,** 1449-1451.

Tamang JP, Tamang B, Schillinger U, Franzcm, Gores M, Holzapfel WH. 2005. Identification of predominant lactic acid bacteria isolated from traditionally fermented vegetable products of the Eastern Himalayas. International journal of food microbiology **105,** 347-356.

Thomson S, Hildebrand D, Schroth M. 1981. Identification and Nutritional Differentiation of the Erwinia Sugar Beet Pathogen from Members of Erwinia carotovora and Erwinia chrysanthemi. Phytopathology **71,** 1037-1042.

Van der Wolf J, De Haan E, Kastelein P, Krijger M, De Haas B, Velvis H, Van Der Zouwen P. 2017. Virulence of Pectobacterium carotovorum subsp. brasiliense on potato compared with that of other Pectobacterium and Dickeya species under climatic conditions prevailing in the Netherlands. Plant Pathology **66(4),** 571-583.

Yusuf I, Oyaweye O, Yongabi K, Pemu A. 2004. Bacteriological Quality Assessment of Salad Vegetables sold in Bauchi Metropolis. Nigeria Journal of Microbiology **18,** 316-320.