International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 2 Number 10 (2013) pp. 83-89**
http://www.ijcmas.com

Review Article

Erwinia carotovora associated with Potato: A Critical Appraisal with respect to Indian perspective.

Rajneesh Prajapat[1], Avinash Marwal[1], Prabhat Nath Jha[2]*

[1]Department of Science, Faculty of Arts, Science and Commerce, Mody Institute of Technology and Science (Deemed University), Lakshmangarh, Sikar - 332311, Rajasthan, India
[2]Department of Biological Science, Birla Institute of Technology & Science (Deemed University) Pilani - 333031, Rajasthan, India
Corresponding author

A B S T R A C T

Keywords

Erwinia carotovora; Potato; disease management.

Erwinia carotovora, an important gram-negative, rod-shaped bacteria (Family *Enterobacteriaceae*), causes soft rot diseases on wide variety of crop species, chiefly potato which is characterized by blackleg of potato plants and soft rot of its tubers during the storage condition, thus causing extreme yield losses worldwide. The aim of proposed review is to depicts the current status and analyze the molecular diversity of *E. carotovora*, recovered from potato and highlighting the various techniques or methods used for *Erwinia* diseases management.

Introduction

Agriculture is demographically a broadest economic sector, plays a significant role in the overall socio-economic development of India. However, productivity of various crops is greatly affected due to infestation of diseases caused by microorganisms. More than 100 known bacterial species are able to cause plant disease. Bacterial diseases are much more prevalent in tropical and sub-tropical regions of the India (*Jackson, 2009)*. A number of preventive measures including chemical treatments are in use to fight against harmful disease caused by bacteria. Different chemical compounds are utilized by poor Indian farmers to overcome the problem of bacterial infection. However, most of these compounds are not target specific and could affect beneficial bacterial population associated with the plant. Moreover, some of these bactericidal compounds (*e.g.*, 1,4-naphthoquinones) get accumulated in plant tissues, which in turn causes several health related problems (Banasiuk *et al.*,2012).

The *Enterobacteriaceae* family member causes soft-rotting disease on a wide variety of crop species worldwide including vegetables, flowers and fruits. A consortium of plant cell wall-degrading extracellular enzymes comprising pectate lyase (Pel), polygalacturonase (Peh), protease (Prt), and cellulase (Cel) contribute to its plant virulence (Chatterjee

et al., 1995). Among them, extracellular pectinases, including Pel and Peh, play a crucial role in tissue maceration and cell death (Favey *et al.,* 1992).

Erwinia carotovora: Transmission and Yield losses

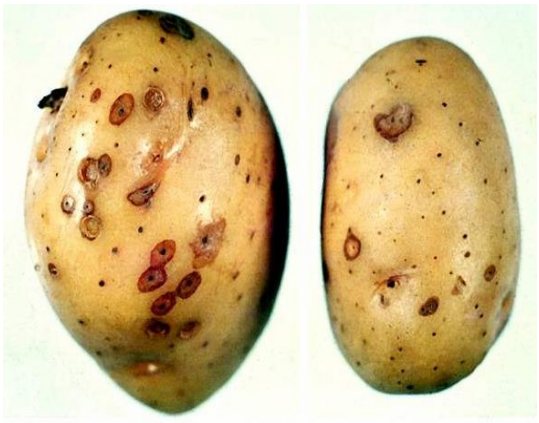
Erwinia carotovora (Family *Entero-bacteriaceae*), is a rod shaped gram-negative phytopathogenic bacterium, and deadliest pathogen which affects productivity of plants (Rogers, 1959). The bacterium causes soft-rot diseases in a variety of plant species including carrots, potatoes, cucumbers, onions, tomatoes, lettuce, mustard and ornamental plants like iris. Transmission of *E. carotovora* occur either through plant to plant or insect to plant. *E. carotovora* causes cell death through plant cell wall destruction by creating an osmotically fragile cell. Yield losses up to 98.8% have been experienced under artificial epiphytotics (Thinda and Payakab, 1985). The commercially important soft rot *Erwinias* are *Erwinia carotovora* subsp. *carotovora* (*Ecc*), *Erwinia chrysanthemi*, and *Erwinia carotovora* subsp. *atroseptica* (*Eca*), which cause diseases of potato and other commercially important crops (Czajkowski *et al.,* 2009; Rahmanifar *et al.*,2012).

E. carotovora is non-sporeforming and peritrichously flagellated. It is a facultative anaerobe, catalase negative and oxidase positive (Harris *et al.*,1998). Direct and indirect crop losses due to these pathogens are considerable, especially in certain production areas (Perombelon and Kelman, 1998).

E. carotovora associated with potato

Potato (*Solanum tuberosum*) is the vegetable listed among the five principal world food crops (FAOSTAT, 2010) and also one of the important food crops in India. The potato crop is generally harvested during February and March in most regions of India. This is a time when temperature starts increasing between 30°C and 40°C in the month of June followed by rains in July and August. Potato in India is cultivated in approx 18-19 lakh hectare which is around 1.25% of total cultivated area in India. It contributed around 2.42% of agriculture GDP from 1.25% cultivated area (SFAC, 2012).

Figure.1 [a] Black leg symptom caused by *Erwinia carotovora* infection, [b] *Erwinia carotovora* subsp. *atroseptica* - Black Leg (Soft Rot) of Potato, [c] Lesions associated with lenticels on a potato tuber caused either by *Erwinia carotovora* pv. *atroseptica*, or *Erwinia carotovora* pv. *carotvora*.



High humidity and temperatures around 30°C favor development of *E. carotovora* infection therefore the climatic conditions are suitable for this pathogen (Colther *et al.*,1983). Disease symptoms observed both outside and inside the tuber. The potato skin look water-soaked and dark blister forms. The bacteria enter through a weak point in the skin and rots out the center of the potato (Fig. 1). Screening of the isolates for antagonistic activity against *E. carotovora subsp. atroseptica* revealed that 38% of the endophytes protected tissue culture plants from blackleg disease (Reiter *et al*., 2002).

Genes that confer soft rot resistance in potato have been identified (gene *ubiquitin7*, or *ubi7*) in tubers (Garbarino and Belknap, 2002), other crops might be also benefited from protection obtainable by *Erwinia* resistance genes linked to the *ubi7* gene's promoter (*Wood, 1998).* A PCR-based method was developed for the simultaneous detection and quantification of the potato pathogen *E. carotovora* subsp. *atroseptica* (*Eca*) on potato tubers. This was the first quantitative PCR-based detection method described for *Eca* and was first for any bacterial plant pathogen to incorporate DNA extraction control (Hyman *et al.,* 2000). Primers ECA1f and ECA2r specifically amplified a 690-bp DNA fragment of all Eca (De Boer and Ward, 1995). Automated conductance measurements in polypectate medium were used for the detection of pathogenic soft rot *Erwinia* spp. in potato peel extracts (Fraaije *et al.*, 1997).

Analysis of *E. carotovora* infection

A PCR-RFLP test based on a pectate-lyase encoding gene permitted the detection of *E. carotovora* that required complete DNA extraction (Helias *et al.,* 1998). Seven monoclonal antibodies (MAbs) against *Eca* have been produced. ELISA-DAS using MAb 4G4 with an enrichment step also efficiently detected *Eca* in naturally infected tubers and plants (Gorris *et al.,* 1994).The relative specificity, sensitivity of recently developed microbiological, immunological and molecular methods to detect and quantify tuber contamination were discussed in relation to the testing of commercial seed stock. (Perombelon *et al.,* 1998). *Prt*W gene provides protease activity for the normal progression of disease symptoms caused by this bacterium (Marits *et al.,* 2002). The *pel*B gene encodes pectate lyase B, one of three pectate lyases identified in *Erwinia carotovora* EC. *Pel*B (*Pel*2) and *Pel*C (*Pel*3) of *Ecc* are considered as the main pectate lyases responsible for plant tissue maceration (Perombelon, 2002). The *hrp* genes encoding a type III secretion system have been considered essential for virulence (De Boer, 2003; Mee-Ngan *et al.,* 2004).

A PCR-based kit, Probelia^{TM}, for the detection of *Eca* on potatoes was evaluated. The kit was based on DNA-specific PCR amplification followed by detection of amplicons by hybridization to a peroxidase-labelled DNA probe in a microplate (Frechon *et al.,* 1998; Darrasse *et al.,*1994). The sensitivity of the PCR for a direct detection of *Eca* in crude peel extracts was only 107-108 cells/ml, due to inhibition of PCR amplification by potato tuber-derived compounds (Fraaije *et al.,* 2008). The 16S-23S rRNA intergenic transcribed spacer (ITS) was used for identification the soft rot *Erwinias*. The ITS was amplified from *Erwinia* and other genera using universal PCR primers (Toth *et al.,* 2001). Serological techniques are also been utilized for identifying the

pathogen *E. carotovora* (De Boer *et al*., 1987). It established that the pathogen perpetuated in the seed, either externally of internally (Prasad and Sinha, 1978). The pathogen produced soft rot in potato, carrot, onion, and also infected jowar (*Sorghum*), bajra [*Pennisetum typhoides*], and tobacco (Hingorani *et al.,*1960).

E. carotovora research at National Status

Reports of the *Erwinia* infection received from various states of India viz., Rajasthan, Madhya Pradesh, Uttar Pradesh and Haryana (Kaushik *et al.,*1973). Seven vegetable species *viz.* carrot (*Dacus carota*), cucumber (*Cucumis sativa*), onion (*Allium cepa*), potato (*Solanum tuberosum*), knol khol (*B. oleracea* var *caulorapa*), cauliflower (*B. oleracea* var *botrytis*), tomato *Lycopersicon esculentum*), belonging to different families were tested for host range studied of the *E. carotovora* in Kashmir (Bhat *et al.,*2010). *Eca* is responsible for 'blackleg' infections in potato plants.

Virulence depends on the production of an arsenal of plant cell wall-degrading enzymes (PCDWEs; including pectinases and cellulases) and many of these virulence determinants, and others, have been found to be under quorum sensing control. Quorum sensing signalling in *Erwinia* uses N-acylhomoserine lactone (AHL)-based systems (Pirhonen *et al.,* 1993) as well as systems that depend on autoinducer-2 (Coulthurst *et al.,* 2006).

Blackleg of tubers incidence is closely linked to the level of *Eca* contamination in seed tubers (Hyman *et al.,* 2000). *E. carotovora* which cause stalk rot of corn survived in soil and host tissues under different conditions (Rangarajan and Chakravarti, 2011). The aqueous extracts of twenty plants were screened by agar diffusion methods for their antibacterial activity against *Eca*, a causal organism of soft rot of potato. The strong inhibitory effect on *Erwinia* species observed by the use of leaf extracts of *Camellia sinensis*, and bark extracts of *Acacia arabicae* and *Acacia catechu* (Bhardwaj and Laura, 2008). L-Asparaginase, an enzyme-drug used for the treatment of acute lymphoblastic leukemia was isolated from *E. carotovora* (Jain *et al.,* 2012).

The effects of different carbon and nitrogen sources on the fermentative production of the enzyme were studied (Maladkar *et al.,* 1993). Nine Phenolic acids were identified in the peel and the pulp of tubers of *Eca* (Kumar *et al*., 1991).Various Isolates of probable antagonistic bacteria of potato soft rot bacterium *Erwinia carotovora* subsp. *carotovora* (*Ecc*) were extracted from rhizospheres and endophytes of various crop plants in the potato farming areas of Bangladesh. The findings suggest that isolate E-65 could be exploited as a biocontrol agent for potato tubers (Rahman *et al.,* 2012).

This review appears to be an accurate, useful tool, in particular for molecular, serological and epidemiological studies. It allows the clear definition of groups that may further solve the controversial question of host specificity in *E. carotovora*. *E. carotovora* is a soft-rot disease causing bacteria and economically very harmful pathogen in terms of post-harvest losses, and a common cause of decay in stored fruits and vegetables. Potato associated *E. carotovora* affects plant health and physiology in a variety of ways that resulted in major economic losses to the potato. The multi-factorial

nature of biological and chemical control could be used for plant diseases management through cellular expression of biocontrol related genes and secretion of bioactive metabolites.

References

Banasiuk, R., A. Kawiak and Królicka A. 2012. *In vitro* cultures of carnivorous plants from the *Drosera* and *Dionaea* genus for the production of biologically active secondary metabolites. J. Biotech, Computational Bio. Bionanotech. 93(2): 87-96.

Bhardwaj, S. K. and Laura J. S. 2008. Antibacterial activity of some plant extracts against pathogenic bacteria *Erwinia carotovora* Subsp. *Carotovora*. Potato Journal. 35: 72-77.

Bhat, K. A., N. A. Bhat, S. D. Masoodi, S. A. Mir, M. Y. Zargar and Sheikh P. A. 2010.Studies on status and host range of soft rot disease of cabbage (*Brassica oleracea* var *capitata*) in Kashmir valley. J. of Phytol. 2: 55–59.

Colther, E. J. and Powell V. 1983. Physiological and pathological characteristics of *erwinia chrysanthem* isolated from potato tuber. J. App. Bacteriol. 54: 37-43.

Chatterjee, A., Y. Cui, Y. Liu, C. K. Dumenyo and Chatterjee A. K. 1995. Inactivation of *rsmA* leads to overproduction of extracellular pectinases, cellulases, and proteases in *Erwinia carotovora* subsp. *carotovora* in the absence of the tarvation/cell density-sensing signal, N-(3-oxohexanoyl)-L-homoserine lactone. Appl. Environ. Microbiol. 61: 1959-1967.

Coulthurst, S. J., K. S. Lilley and Salmond G. P. C. 2006. Genetic and proteomic analysis of the role of luxS in the nteric phytopathogen, *Erwinia carotovora*. Mol. Plant Pathol. 7: 31–45.

Czajkowski, R., G. Grzegorz and Van Der Wolf J. M. 2009. Distribution of Dickeya spp. and *Pectobacterium carotovorum* subsp. carotovorum in naturally infected seed potatoes. Eur. J. Plant. Pathol. 125: 263-275.

Darrasse, A., A. Kotoujansky and Bertheau Y. 1994. Isolation by genomic subtraction of DNA probes specific for *Erwinia carotovora* subsp. *atroseptica*. Appl. Environ. Microbiol. 60 :298-306.

De Boer, S. H. 2003. Characterization of pectolytic erwinias as highly sophisticated pathogens of plants. Eur J Plant Pathology. 109: 893-899.

De Boer, S. H. and Ward L. J. 1995. PCR Detection of *E. carotovora* subsp. *atroseptica* associat ed with Potato Tissue. Phytopathology. 85: 845-58.

De Boer, S. H., L. Verdonck, H. Vruggink, P. Harju, H. O. Bång and Ley J. D. 1987. Serological and biochemical variation among potato strains of Erwinia carotovora subsp. atroseptica and their taxonomic relationship to other E. carotovora strains. J Appl Bacteriol. 1987; 63:487–495.

FAOSTAT, 2010.FAOSTAT. Available at: http://faostat.fao.org/site/339/default .aspx.

Favey, S., C. Bourson, Y. Bertheou, A. Kotoujansky and Boccora M. 1992. Purification of the acidic pectate lyase of Erwinia chrysanthemi 2942 and sequence analysis of the corresponding gene. J. Gen. Microbiol. 138: 499-508.

Fraaije, B. A., M. Appels, S. H. De Boer, J. W. L. Van Vuurde and Van den Bulk R. W. 1997. Detection of soft rot Erwinia spp. on seed potatoes: conductimetry in comparison with

dilution plating, PCR and serological assays. European J. Plant Pathol. 103:183-193.

Fraaije, B. A., Y. Birnbaum and VanDen Bulk R.W. 2008. Comparison of Methods for Detection of *Erwinia carotovora* ssp. *atroseptica* in Progeny Tubers Derived from Inoculated Tubers of Solanum tuberosum L. J. of Phytopathol. 144: 551–557.

Frechon, D., P. Exbrayat, V. Helias, L. J. Hyman, B. Jouan, P. Llop, M. M. Lopez, N. Payet M. C. M. Pérombelon, I. K. Toth, *et al*, 1998. Evaluation of a PCR kit for the detection of *Erwinia carotovora* subsp. *atrosepticaon* potato tubers. Potato Res. 41:163-173.

Garbarino, J. E. and Belknap W. R. 2002. Ubiquitin-lytic peptide fusion gene constructs, protein products derived there from, and methods of making and using same. Ubi7 promoter patent: U.S. Patent 414102, S/N 091,482, 611.

Gorris, M. T., B. Alarcon, M. M. Lopez and Cambra M. 1994. Characterization of Monoclonal Antibodies Specific for *Erwinia carotovora* subsp. *atroseptica* and Comparison of serological methods for its sensitive detection on potato tubers. Appl. Environ. Microbiol. 60: 2076-2085.

Harris, S. J., Y. L. Shih, S. D. Bentley and Salmond G. P. 1998. The hexA gene of Erwinia carotovora encodes a LysR homologue and regulates motility and the expression of multiple virulence determinants. Mol Microbiol. 28:705-17.

Helias, V., A. C. Le Roux, Y. Bertheau, D. Andrivon, J. P. Gauthier and Jouan B. 1998. Characterisation of *Erwinia carotovora* subspecies and detection of *Erwinia carotovora* subsp atroseptica in potato plants, soil and water extracts with PCR-based methods. European J. Plant Pathol. 104: 685-699.

Hingorani, M. K., U. J. Grant and Singh N. H. 1960. *Erwinia carotovora* .sp. *zeae*, a destructive pathogen of Maize of India. Indian Phytopathology. 12: 151-157.

Hyman, L. J., P. R. Birch, A. Dellagi, A. O. Avrova and Toth I. K. 2000. A competitive PCR-based method for the detection and quantification of *Erwinia carotovora* subsp. *atroseptica* on potato tubers. Lett Appl Microbiol. 4:330-5.

Jackson, R. W. 2009. Plant Pathogenic Bacteria: Genomics and Molecular Biology. Caister Academic Press. ISBN 978-1-904455-37-0.

Jain, R., K. U. Zaidi, Y. Verma and Saxena P. 2012. L-Asparaginase: A promising enzyme for treatment of acute lymphoblastic leukiemia. People's J. Scientific Res. 5(1): 29-35.

Kumar, A., V. S. Pundhir and Gupta K. C. 1991. The role of phenols in potato tuber resistance against soft rot by *Erwinia carotovora* ssp. *Carotovora*. Potato Res. 34: 9-16.

Maladkar, N. K., V. K. Singh and Naik S. R. 1993. Fermentative production and isolation of L-asparaginase from *Erwinia carotovora*, EC-113. Hindustan. Antibiot. Bull. 35:77-86.

Mee-Ngan, Y., D. B. Jeri and Charkowski A. O. 2004. Genomic Diversity of Erwinia carotovora subsp. carotovora and Its Correlation with Virulence. Appl. Environ. Microbiol. 70(5): 3013–3023.

Perombelon, M. C. M. 2002. Potato diseases caused by soft rot erwinias: an overview of pathogenesis. Plant Pathol. 51: 1-12.

Perombelon, M. C. M., Y. Bertheau, M. Cambra, D. Frechon, M.M. Lopez, *et al*, 1998. Microbiological, immunological and molecular methods suitable for commercial detection and

quantification of the blackleg pathogen, Erwinia carotovora subsp. atroseptica, on seed potato tubers: A review. Bulletin OEPP. 28:141-155.

Pirhonen, M., D. Flego, R. Heikinheimo and Palva E. T. 1993. A small diffusible signal molecule is responsible for the global control of virulence and exoenzyme production in the plant pathogen *Erwinia carotovora*. EMBO J. 12: 2467–2476.

Prasad, M. and Sinha S. K. 1978. Serologic studies of the maize stalk rot pathogen *Erwinia carotovora* f. sp. *zeae*. Zentralbl Bakteriol Naturwiss. 133: 80-85.

Rahmanifar, B, N. Hasanzadeh, J. Razmi and Ghasemi A. 2012. Genetic diversity of Iranian potato soft rot bacteria based on polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. African J. Biotec. 11(6): 1314-1320.

Rangarajan, M. and Chakravarti B. P. 2011.Studies on the survival of corn stalk rot bacteria. Appl. Microbiol. Biotechnol. 33 :140-144.

Reiter, B., U. Pfeifer, H. Schwab and Sessitsch A. 2002. Response of endophytic bacterial communities in potato plants to infection with Erwinia carotovora subsp. *atroseptica*. Appl Environ. Microbiol. 68(5):2261-8.

Rogers, M. A. 1959. Decay of poinsettia cuttings by the soft rot bacterium, *Erwinia carotovora* (Jones) Holland. Plant Dis. Rep. 43:1236-1238.

SFAC, 2012. SFAC. Available at: http://www.sfacindia.com/PDF/SFAC _Value-Chain-Analysis.pdf

Thinda, B. S. and Payakab M. M. 1985. A review of bacterial stalk rot of maize in India. Trop. Pest Manage. 31:311-316.

Toth, I. K., A. O. Avrova and Hyman L. J. 2001. Rapid Identification and Differentiation of the Soft Rot Erwinias by 16S-23S Intergenic Transcribed Spacer-PCR and Restriction Fragment Length Polymorphism Analyses. Appl. Environ. Microbiol. 67(9): 4070–4076.

Vir, S., C. D. Kaushik and Chand J. N. 1973. The occurrence of bacterial rot of raya (*Brassica juncea*) in Haryana. Int. J. Pest. Management. 19: 46-47.

Wood, M., Research Service Information Staff, 800 Buchanan St., Albany, CA 94710. http://www.ars .usda.gov/is/ar /archive/jan98/tool0198.htm?pf=1.