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A review on bacterial stalk rot disease of maize caused by *Dickeya zeae*

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Abstract: Bacterial stalk rot of maize caused by *Dickeya zeae* previously known as *E. chrysanthemi* pv. *zeae* have economic importance of reduced crop yield up to 98.8%. The disease is more prevalent in rainy season in India. The bacterium prefers high temperature and moisture for their growth result is plant toppled down within week. The pathogen has wide host range (maize, rice, tomato, chilli and brinjal etc.) which help to pathogen for long survival in soil. The bacterium characterized by biochemical and molecular tactics. In present, *Pel* gene and rDNA specific primers are frequently used for *D. zeae* characterization. The pathogen significantly controls under *in vitro* and *in vivo* condition via bleaching powder (drenching of 100 ppm) and antibiotics. The present studies generated data on pathogen nomenclature, etiology, epidemiology, host range, pathogen survival, biochemical, physiological and molecular characterization, germplasm evaluation and disease management.

Keywords: Bacterial stalk rot, Crop yield, Disease, *Dickeya zeae,* Maize

INTRODUCTION

Maize is the third largest planted crop after wheat and rice in the world (USDA 2014). Production of maize is constrained by a number of abiotic (unfavorable climate like high and low temperature; nutritional imbalance) and biotic factors such as mycoplasma, nematode, fungi and bacteria (Jugenheimer, 1976). Among the biotic factors the diseases caused by fungi and bacteria are economically more important because they cause heavy yield losses 8.5% (Oerke, 2006). During the recent years bacterial stalk rot disease has emerged as one of the most important disease in *kharif* sown maize crop in India (Kumar *et al.,* 2015a). The *Kharif* sown crop has the most susceptible stage coinciding with the annual monsoon rainfall, which aggravates the disease development. Bacterial stalk rot disease was reported for the first time by Prasad (1930), who identified the bacteria involved as *E. dissolvens* but the symptoms described by him resembled more closely to those incited by *E. chrysanthemi* pv. *zeae*. Its importance was realized during 1969 season, when a severe outbreak occurred in Mandi district in Himachal Pradesh. The pathogen spreads from plant to plant and field to field through rainwater and its runoff. The infestation of the disease was described in various parts of the world (Hingorani *et al*., 1959; Pauer, 1964; Prasad, 1930; Sabet, 1954; Volcani, 1961; Zachos *et al*., 1963; Martinez-Cisneros *et al.,* 2014). Three bacterial pathogens have been reported to cause stalk rot of maize namely, *E. dissolvens, E. chrysanthemi* pv. *zeae* and *Pseudomonas syringae* pv. *lapsa* (Prasad 1930; Hingorani *et al.,* 1959; Sinha, 1966). The pathogen has been recently re-classified as *D. zeae* by (Samson *et al.,* 2005). The survey generated 458 votes from the International Community, and allowed the construction of a top 10 bacterial plant pathogen, in which *Dickeya* spp. found 9th place (Mansfield *et al*., 2012). This bacterium has a wide host range causing soft rot (Bradbury, 1986) which make it difficult to manage this bacterium (Goto, 1979). Maize plant toppled down under severe conditions and foul odor emerges. The disease is causing causing severe grain yield losses which can range from 21 to 98 per cents (Thind and Payak 1978).

Favorable environmental conditions: *Dickeya zeae* is preferred high temperatures and high relative humidity for infection and disease development. High temperature and humidity important for physiological and metabolic activity of bacterium therefore its growing well and producing sufficient pectolytic enzymes which is important for plant cell degradation. It can be a problem with areas of heavy rainfall or where overhead irrigation is used and the water is pumped from a lake, pond, or slow-moving stream. Prasad and Sinha (1980) studied that a temperature of 35°C, 70% RH (relative humidity) and inoculum level of 2 x 10^8 cells/

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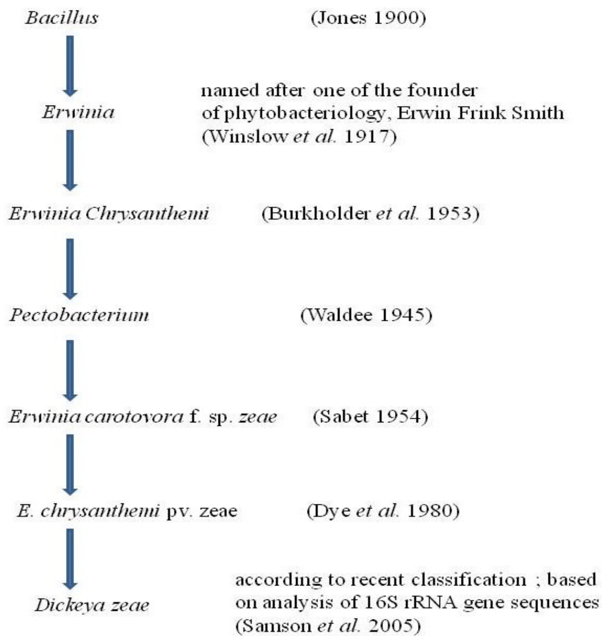
Nomenclature of the pathogen *D. zeae*



ml were essential for disease development in 15 to 30 day old maize plants. Saxena and Lal (1984) made an attempt to correlate weather parameter to the disease and found that temperature and RH did not fluctuate much during all the crop seasons. However, a significant difference was in total rainfall and duration of 'bright sunshine was observed. Saxena and Lal (1981) also studied positive correlation of disease with high nitrogen fertilizer.

Morphology: *D. zeae* is a motile, gram-negative, rod shaped bacterium. It is varying from 0.8-3.2 x 0.5-0.8 µm (average 1.8 x 0.6 µm). There are 3-14, but more usually 8-11, peritrichous flagellae. The bacterium is produced off white, slimy and shiny colonies on King's B Medium (Fig. 1A and B) (Kumar *et al.* 2015b).

Pathogen mode of infection and symptoms: Initial disease symptoms include discoloration of the leaf sheath, which spread further to stalk, leaves and plant topples down in severing condition and a foul odor is detected (Fig. 2A and B). The first stage of maceration by *E. chrysanthemi* involves the entry of the bacteria to the parenchymatous tissues of plants that have been physiologically compromised, such as by bruising, excess water or high temperature (Collmer and Keen 1986). The next stage involves local maceration as a result of depolymerization of plant cell walls, followed by necrosis of the entire plant (Barras *et al.* 1994). Due to the complexity of plant cell walls, which consists of polysaccharides, the main ones being cellulose, hemicellulose and pectin, a variety of enzymes are accordingly produced by *E. chrysanthemi* for the efficient breakdown of cell walls (Robert-Baudouy *et al.* 2000). The major enzymes have been found to be pectinases which degrade various components of pectin using different reaction mechanisms. Other hydrolytic enzymes are also produced, such as cellulase isozymes, protease isozymes, xylanases and phospholipases (Collmer and Keen 1986; Hugouvieux-Cotte-Pattat *et al.,* 1996; Kothari and Baig, 2013; Nahar *et al.,* 2015). It has also been reported that *E. chrysanthemi* is capable of causing systemic disease by spreading through the vascular system of a plant. The physiological symptoms of such infection are yellowing of new leaves, wilting and a mushy, foul smelling stem rot (Slade and Tiffin, 1984). Genetic and physiological studies show that systemic infection of *E. chrysanthemi* is dependent on two abilities namely, iron acquisition and production of the pigment, indigoidine (Expert and Tousaint 1985; Reverchon *et al.,* 2002). Due to iron scarcity in the environment and its role as an essential element, most organisms have derived the ability to sequester iron by production of low-molecular-weight high affinity iron-chelating agents called siderophores. These are produced in response to iron limitation in order to capture $Fe3+$ ions. In a plant -bacteria interaction, the successful competition for iron between the two organisms could determine the outcome of an invasion (Enard *et al.,* 1988).

Similarities to other diseases: Pythium stalk rot (*Pythium aphanidermatum*) causes similar symptoms on maize, but bacterial stalk rot may be accompanied by a foul odour.

Host range: *D. zeae* bacteria have a wide host range. Bradbury (1986) reported that *E. chrysanthemi* is causal agent of soft rot disease on wide range of plant species in tropical, subtropical and temperate region of the

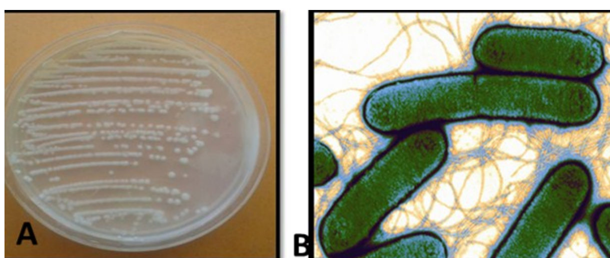


Fig. 1. A, B. *Purified single colony culture of Dickeya zeae on King's B agar plate (Kumar 2015b). Electron microscopic image of Pectobacterium atrosepticum (James Hutton Institute, 2017), a species closely related to D. zeae.*

Fig. 2. A, B. *Symptoms of bacterial stalk rot produced by D. zeae (Kumar, 2015).*

world. It attacks tubers of potato and sweet potato, onion bulbs, bean pods, roots of carrot, turnip, radish and sugar beet, fruits of tomato, brinjal, chillies and papaya and plants of pearl millet, sorghum, brinjal, potato, tomato, tobacco and cabbage (Thind, 1970; Rangarajan and Chakravarti, 1971; Hingorani *et al*., 1959; Mehta, 1973; Sinha and Prasad, 1977). Goto (1979) reported that *E. chrysanthemi* caused bacterial foot rot disease of rice in Japan. Similarly, Qiongguang and Zhenzhong (2004) reported foot rot disease of rice in China caused by *E. chrysanthemi* pv. *zeae.* Edward *et al.* (1973); Lakshmanan and Mohan (1980); Khan and Nagaraj (1998) reported tip-over of banana caused by *E. carotovora* subsp. *carotovora* and *E. chrysanthemi* from across the world. In India it was reported to be caused by *E. carotovora* subsp. *carotovora* (Edward *et al.* 1973; Lakshmanan and Mohan 1980; Khan and Nagaraj, 1998), while Chattopadhyay and Mukherjee (1986) attributed it to be *E. chrysanthemi*. Bacterial heart rot of the pineapple caused by *E. chrysanthemi* was first reported on Malaysia (Johnston 1957) and has since been described in Costa Rica (Chinchilla *et al.,* 1979), Brazil, and the Philippines (Rohrbach and Johnson 2003). *Erwinia chrysanthemi* bacterium is also known as a greenhouse pathogen in mild climate regions (Perombelon and Kelman 1980). Stem rot caused by *E. chrysanthemi* on tomato in greenhouses has been first reported on Turkey (Cinar and Aysan, 1995). Recently, Kumar *et al*. (2015a) studied that *D. zeae* populations of Punjab have wide host range and cross-infecting many hosts (Fig. 3).

Survival: The soil represents a favorable habitat for microorganisms and is inhabited by a wide range of microorganisms, including bacteria, fungi and protozoa. *D. zeae* survives in plant debris but the survival period varies from different environmental conditions (Anil Kumar and Chakravarti, 1971b; Prasad and Sinha 1977; Saxena and Lal 1982). The best soil compostion for *D. zeae* growth is low population of PGPR (Plant growth-promoting rhizobacteria) with infected maize debris in soil. Anil Kumar and Chakravarti (1971b) studied that bacterium survived for 24, 15 and 12 weeks in infected tissue (40% soil moisture) at 10, 20 and 30 °C and for 18, 15, 12 and 12 weeks (kept in soil at 27 °C) at 98, 95, 90 and 81% relative humidity (RH), respectively. However, population of the bacterium was reduced at >90% moisture, due to decreases rates of organic matter decomposition, due to low oxygen supply (Csonka 1989; Killham *et al.,*1993). Seed survival of the bactertium which artificially inoculated also studied by Anil Kumar and Chakravarti (1971a), they found the bacterium survived for 5 months at 10 and 20 °C with 81 and 93% RH and for 3-4 months at 30 and 35 C with 51 and 62% RH. The bacterium survived for 140 days in autoclaved soil at 40% moisture compared to only 29 days in non-autoclaved soil (Anil Kumar and Chakravarti, 1970).

However, Rangarajan and Chakravarti (1970b) studied that stalk rot bacterium survived for 150 and 90 days in sterile and unsterile soils, respectively. Prasad and Sinha (1977) found that a sterilized environment increased the survival period of the bacterium in comparison to an unsterilized environment. It survived for 3-4 months in soil alone and for 4-6 months in soil containing healthy maize stalks. The survival period was longest (9 months) in soil which contained naturally and artificially infected maize plants as debris. Saxena and Lal (1982) also reported the longer survival period in sterilized soils and heavier soils. The maize borer, Chilo partellus, was shown to act as a carrier of this bacterium. It spreads the pathogen from diseased to healthy plants (Thind and Singh, 1976). Recently Kumar *et al.* (2017) studied on survival on the bacterium *in vivo* and *in vitro* condition at Punjab Agricultural University, Ludhiana. Highest survival of the pathogen (270 days) was found in both type of soils field and sterilized soil (autoclaved soil) when mixed with host (maize) debris. The period of survival was positive correlated with increase in moisture and was maximum at 90%. The pathogen showed highest log cfu/ml at 30 ^{o}C and store

Table 1. Statistics for the 12 draft *Dickeya* genome sequences.

Species	Strain	Accession no.	No. of Contigs	No. of assembled bases	N50	No. of predicted coding sequences
D. chrysanthemi	NCPPB 402^{T}	AOOA00000000	12	4,797,070	2,467,266	4,447
D. chrysanthemi	NCPPB 516	AOOC00000000	35	4,614,776	443,362	4,444
D. chrysanthemi	NCPPB 3533	AOOJ00000000	91	4,723,912	102,359	4,467
D. dadantii	NCPPB 898^{T}	AOOE00000000	52	4,933,637	191,282	4,591
D. dadantii	NCPPB 2976^{T}	AOOG00000000	84	4,810,532	114,781	4,552
D. dadantii	NCPPB 3537	AOOL00000000	47	4,805,222	222,170	4,430
D. zeae	NCPPB 2538^{T}	AOOF00000000	46	4,559,915	237,408	4,225
D. zeae	NCPPB 3531	AOOI00000000	29	4,623,158	385,197	4,256
D. zeae	NCPPB 3532	AONW00000000	19	4,555,162	330,312	4,261
D. zeae	CSL RW192	AONY00000000	56	4,696,643	240,868	4,402
D. zeae	MK19	AOOR00000000	35	4,669,100	417,168	4,346
D. paradisiacal	NCPPB 2511^{T}	AONV00000000	43	4,627,470	160,099	4,376

Fig. 3. *Symptoms produced by D. zeae on a. tomato (Punjab Varkha bahaar-1); b. rice (Pusa 44); c. brinjal (Punjab Sadabahaar); d. maize (Punjab Sweet Corn-1); e. chilli (Punjab Lal Surkh); f. maize (Double Dekalb) (Kumar 2015).*

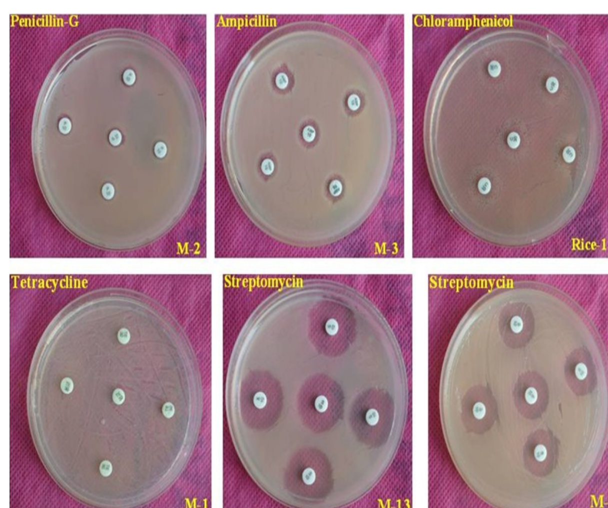


Fig.4. *Sensitivity of five different antibiotics against six isolates of D. zeae using HiMedia® antibiotics discs. Except for streptomycin all the other four antibiotics were ineffective against the test isolates (Kumar 2015).*

viability of *D. zeae* was 36 months (3 years) in silica gel, while virulence of the pathogen exists only one maize crop season.

Biochemical and physiological characterization of *D. zeae*: Biochemical tests help to identification of different bacterial species based on the differential biochemical activities. Differences in carbohydrate, protein, fat metabolism, production of certain enzymes, ability to utilize a particular compound etc, help to be identifying the microorganisms.

Hingorani *et al.* (1959) studied 6 isolates collected from India and classified them as *E. chrysanthemi* pv. *zeae*, whereas Rangarajan and Chakravarti (1967) isolated bacteria from maize variety Ganga-3 and identified the bacterium as *Pseudomonas lapsa*. Furthermore, Dickey (1979) identified 421 strains of *Erwinia species.* All strain of *E. chrysanthemi* were separated from the others *Erwinia* species primarily by three physiological characters such as production of gas from D-glucose, phosphatase production and inability to produce acid from D-trehalose. The 322 strains of *E. chrysanthemi* were separated into five infrasubspecific subdivisions based on physiological properties.

Thind and Payak (1979) studied the motility and virulent characters of *E. chrysanthemi* pv. *zeae* with help of a medium containing 2, 3, 5-triphenyl tetrazolium bromide TTB (2, 3, 5-triphenyl tetrazolium bromide), the colony characters of cell motility and pectolytic activity can be used to differentiate virulent and avirulent cultures. Virulent types produce larger and deeper wells as compared to avirulent ones on sodium polypectate medium. Virulent cultures showed abundant motility in hanging drop and in semi-solid medium and possessed numerous flagella. While, the avirulent cells were show poor motility with few flagella, small red centres and wide colourless borders colonies on TTB.

Saxena and Lal (1982) found that bacterial cells suspended in sterile water and stored at 5 °C or less remained virulent for 2 years. However, Kumar *et al.* (2017) observed that the virulence of the pathogen exists only one crop season but it can survive 3 years in silica gel.

Henz *et al.* (2006) identified 227 isolates of *Erwinia* spp. by biochemical and physiological tests (pectolytic activity, lecithinase, α-methyl glucoside, phosphatase, erythromycin sensivity, growth at 37°C) from arracacha roots out of which 89.9% isolates were *E. chrysanthemi*, 9.7% as *E. carotovora* subsp. *carotovora* and 0.5% as *E. carotovora* subsp. *atroseptica.* Furthermore, Kaneshiro *et al.* (2008) studied 48 strains of *E. chrysanthemi*, isolated from pine apple infected plant and irrigation water. Out of 48 isolates, 33 isolates were gram-negative, fermented glucose, formed pits on Crystal Violet Pectate (CVP) medium, reacted with MAb E2, and produced beige and flat colonies of dry consistencies on Yeast dextrose chalk agar (YDC) medium therefore, were presumptively identified as *Erwinia* species. Twenty two strains isolated from plants originally imported from Costa Rica and Honduras and 1 strain from Hawaiian irrigation water were also positive for both indigoidine and indole, suggesting that they were *E. chrysanthemi*. Seven of the remaining *Erwinia* strain were fermentative and pectolytic but negative for indigoidine and indole production, suggesting an *E. carotovora* identification. Nine *Erwinia* spp. strains isolated from banana were identified on the basis of morphological, cultural, physiological, biochemical characteristics and pathogenicity tests. Seven isolate I1 to I6 and I8 showed similarities to *E. carotovora subsp. carotovora*. Isolate I9 from Andhra Pradesh expressed characteristics similar to that of *E. chrysanthemi* and was identified as *E. chry-*

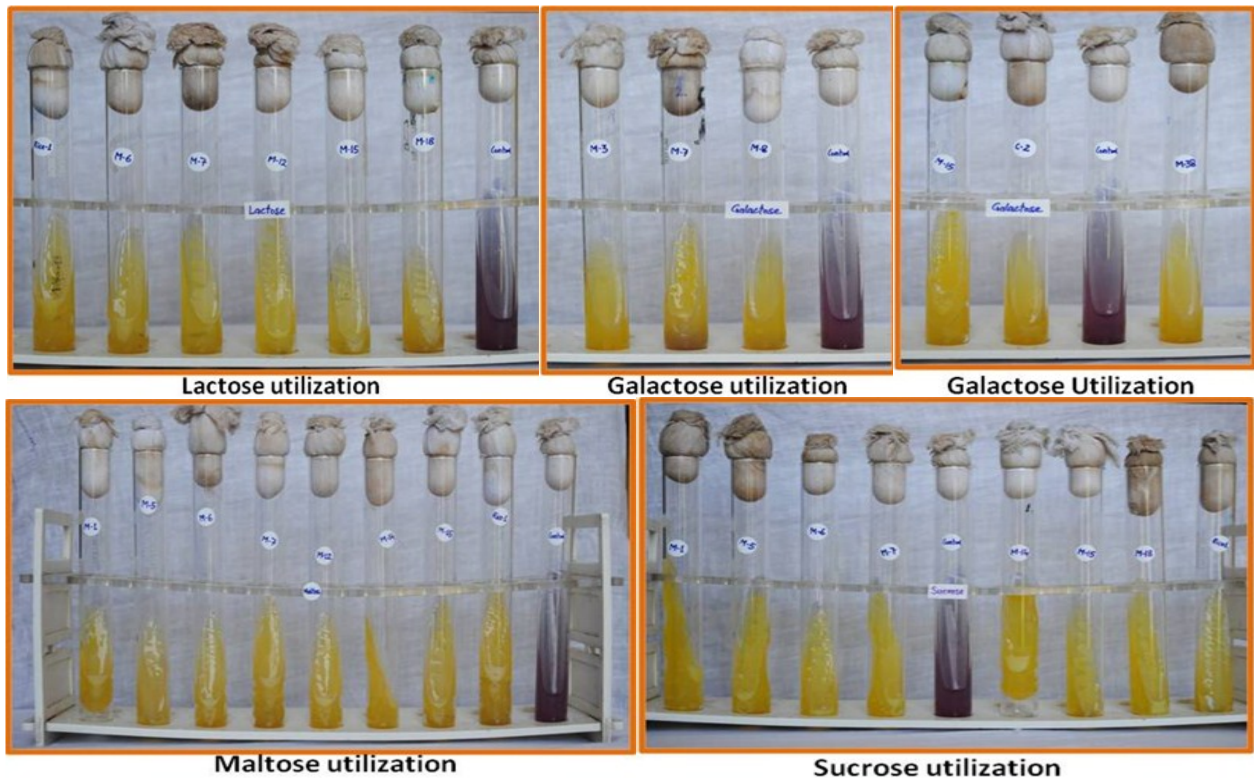


Fig. 5. *Sugar utilization by different maize isolates of D. zeae (Kumar 2015).*

santhemi. The isolate I7 which showed wider variation, neither confirmed to the characteristics of *E. carotovora* subsp. *carotovora* nor with that of *E. chrysanthemi*, nor possessed characteristics in between the two species (Snehalatharani and Khan 2010). Currentely, Kumar *et al*. (2015b) used 27 biochemical tests to characterize 59 isolates of *D. zeae* which showed differential reaction to utilization of carbohydrates, gelatin liquefication and growth at high salt concentration (Fig. 5). Multiple antibiotic resistances were also observed in all the isolates tested (Fig 4).

Pathotypic characterization of *D. zeae*: *E. chrysanthemi* is a phytopathogenic bacterium which induces soft rot and wilting Burkholder *et al.* (1953). The bacterium attacks a wide range of host-plants, and occurs in many areas of the world (Bradbury 1986). In phytobacteriology, infra-subspecific epithets were chosen as "pathovars" terms currently used to designate organisms on the basis of their host range (Young *et al.* 1978). *E. chrysanthemi* was first divided into 4 pathovars from the host *Chrysanthemum morifolium*, *Dieffenbachia* spp., *Parthenium argentatum* and *Zea mays*. Then 2 more pathovars were added namely pv. *dianthicola* from *Dianthus* sp and pv. *paradisiaca* from *Musa paradisiaca*. Pathovars are listed in the last Bergey's Manual of Systematic Bacteriology (Lelliott and Dickey 1984) with the mention that "the relationship between phenotypic, pathogenicity properties and serological reactions of strains of the pathovars is not entirely clear."

Cother and Powell (2008) studied nine isolates of *E. chrysanthemi* isolated from rotted potato tubers compared with 6 exist strains. Phenotypic properties of the potato isolates closely agreed with those of *E. chrysanthemi* pv. *zeae* and with the characteristics proposed for Dickey's infrasubspecific subdivision IV (1979) and Samson and Nassan-Agha's biovar 3 (1978), where *Zea mays* be among the most common host species. Koch's Postulate tests on twenty ornamental and agricultural species showed only *Cyclamen* sp. and *Zeae mays* to be susceptible. In ODD (Ouchterlony double diffusion) tests, antisera to whole live cells of one potato strain reacted with four of the six pathovars of *E. chrysanthemi*. Tuber isolates did not produce blackleg symptoms in inoculated stems. Furthermore Ali *et al.* (2013) studied 20 isolates of *E. carotovora* sub sp. *atroseptica* causing blackleg of the potato collected from Pakistan. Pathogenicity tests divided these 20 isolates into 4 aggressiveness groups or AGs. AG 1 (7 isolates) group was most aggressive causing an average of 5.69 cm rot on the potato stem.

Molecular characterization of *D. zeae*: Molecular characterization is an important tool for identification of plant pathogens with help of locus/gene specific primers. *D. zeae* bacterium has wide host range due to plant cell wall degrading enzymes (pectate lyase), which are important virulent factor (Barras *et al.,* 1994; Salmond, 1994).

Darrasse *et al.* (1994) used *pel* gene sequence to identify *E. carotovora* and they were observed that tested

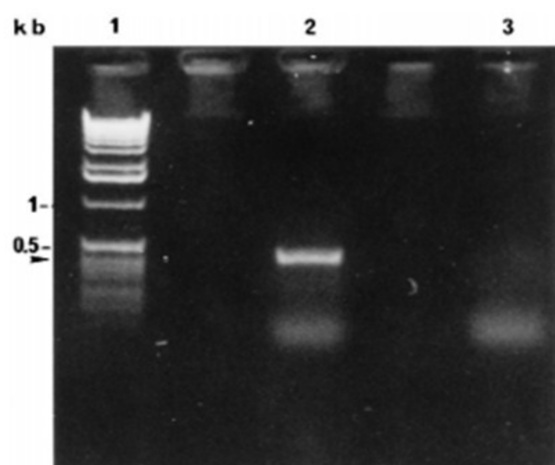


Fig. 6. *PCR amplification of pelADE fragments with primers ADE1 and ADE2. The PCR products (after 25 cycles) were separated by electrophoresis on a 1% agarose gel. Lane 1, 1 -kb DNA ladder; lane 2, E. chrysanthemi 3937. The arrowhead indicates the position of the 420-bp amplified fragment (Nassar et al., 1996)*

isolates (89) present 420 bp bands. Similarly, Nassar *et al.* (1996) developed *E. chrysanthemi* specific primer set (ADE-1, ADE-2) for detection of 78 strains of *E. chrysanthemi* and they observed all starins showed 420 bps specific bands (Fig.6). Similar primers were also used by many autors for detection of that pathogen (Henz *et al.* 2006; Kaneshiro *et al.* 2008). Smid *et al.* (1995) developed ERWFOR and ATROREV gene specific primers and used these for characterization of *E. carotovora* subsp. *atroseptica* and *E. chrysanthemi* in potato. Toth *et al.* (2001) used AFLP fingerprinting to determine the taxonomic relationships within *E. carotovora* and *E. chrysanthemi* groups based on their genetic relatedness. Fessehaie *et al.* (2002) studied molecular characterization of DNA encoding 16S–23S rRNA intergenic spacer regions and 16S rRNA of pectolytic *Erwinia* species. Comparison of 16S rDNA sequences from different species and subspecies clearly revealed intraspecies-subspecies homology and interspecies heterogeneity. Similarly, Slawiak *et al.* (2009) characterized *Dickeya* spp. form potato and two strains of Hyacinthus by using biochemical assays, REP-PCR genomic finger printing, 16S rDNA and DNA X sequence analysis. Furthermore, Ali *et al.* (2013) characterized twenty isolate of *E. carotovora* subspecies *atroseptica* (Eca) causing blackleg of potato, with help of subspecies-specific primers Eca 1F and Eca 2R.

Analysis of whole genome: Genome sequencing of the pathogens an important step to understand the mechanisms of pathogenesis and process of limit host range of the strain. The nucleotide sequence of the genomes of several phytopathogenic bacteria, such as *Agrobacterium tumifaciens, Pseudomonas syringae, Ralstonia solanacearum, Xylella fastidiosa* and two *Xanthomonas oryzae* and many species of soft rot *Erwinia* recently determined (Simpson *et al.,* 2000; Buell *et al.,* 2003; Lee *et al.,* 2005; Salanoubat *et al.,* 2002; Wood *et al.,* 2001; Pritchard *et al.* (2013).

The *Dickeya* genus is recentely described six species: *dianthicola*, *dadantii*, *zeae*, *chrysanthemi*, *paradisiacal* and *solani* (Samson *et al.,* 2005; Brady *et al.,* 2012; Van der *et al.,* 2013). Draft genome sequences of eight *D. dianthicola* and *D. solani* isolates were recently described (Pritchard *et al.,* 2013), and four complete sequences of *Dickeya* strains, *D. paradisiaca* (Ech703), *D. zeae* (Ech586), *D. chrysanthemi* (Ech1591) and *D. dadantii* (Ech3937) have been deposited in GenBank (Glasner *et al.* (2011). Pritchard *et al.* (2013) announced draft genome sequences of 17 isolates of *Dickeya*, including 12 isolates of *D. dadantii*, *D. chrysanthemi*, *D. zeae*, and *D. paradisiaca* (Table 1). Similarly Bertani *et al.* (2013) determined sequence of *D. zeae* (DZ2Q) from diseased rice from a Roma cultivar grown in the Po Valley.

Host-plant resistance: Host plant resistance is the most economic approach to manage this disease. Identification and use of resistance sources in breeding programme have been employed by various researchers (Rangarajan and Chakravarti 1969; Thind and Payak 1976; Ebron *et al.* 1987; Sah and Arny 1990). Complete resistance to this pathogen has not been reported so far, but various authors have tried to identify qualitative traits loci conferring the qualitative/ multigene resistance against bacteria soft rot (Canama and Hautea 2010). Rangarajan and Chakravarti (1969) evaluated 20 maize varieties (4 composite and 16 hybrids) in the field against *E. carotovora* pv. *zeae* (M1 and M2) and observed that all varieties were resistant. Sinha and Prasad (1975) reported partial resistance against *E. chrysanthemi* pv. *zeae* in CM 600, CM 104 and CM 105 maize lines and their crosses in the field. Thind and Payak (1976) reported laboratory method (cut stalk method) for evaluation of maize lines against *E. carotovora* var. *zeae.* They observed that development of disease reaction in both laboratory and field method was similar but with some minor departures. They concluded that 'cut stalk method' can be used for screening maize germplasm.

Thind and Payak (1978) evaluated 32 maize entries consisting of 13 inbred lines, 9 hybrids, 6 composites and 4 open pollinated varieties against *E. chrysanthemi* pv. *zeae*. They observed that two inbred lines CM-101, CM- 110 and two open pollinated varieties CM-600, Basi were found to be tolerant against *E. chrysanthemi* pv. *zeae*. Sinha and Prasad (1981) reported that susceptibility of maize varieties was due to enhanced proteolytic enzyme activity and change in protein and total amino acid contents of stalk and leaf tissues of plant in middle and old age of crop. However, Srivastava and Prasad (1981) observed that the susceptibility of maize plants was dependent on the induction of cellulose activity by the bacterium in the infected

tissues. Ebron *et al.* (1987) evaluated 107 maize accessions against bacterial stalk rot during the wet season of 1985 and 208 during the dry season of 1986. Inoculation of test material was done by whorl inoculation techniques after 30-32 days of emergence. The percentage of bacterial stalk rot toppling were very high in both seasons of 1985 and 1986, only 8 entries out of 107 and 30 entries out of 208 maize accessions were considered resistant. Sah and Arny (1990) evaluated 45 cultivars against *E. chrysanthemi* pv. *zeae* and found a significant positive correlation between field and green house tests. Arun cultivar showed the lowest disease incidence (39.2%), while the highest were recorded in PI 165982 cultivar (94.6%). Maize hybrids and open-pollinated varieties inoculated with *E. chrysanthemi* appeared to possess genes for resistance that can be accumulated through appropriate selection techniques (Dionio and Raymundo 1990).

Control of bacterial stalk rot disease: Bacterial stalk rot disease of maize has been managed by employing several methods such as cultural practices, biological and chemical control.

Cultural practices: The pathogen infection can be suppress via organic manure amendment which stimulates the population of beneficial microflora and avoid flooding and excessive irrigation. Ridge sowing method also helps to the farmer to manage that disease. Kumar *et al.* (2015c) were survey maize growing areas of Punjab and found minimum disease incidence and severity as compared to flat sown method in the farmer field.

Chemicals management: The use of many chemicals to control of *E. chrysanthemi* pv. *zeae* under *in vitro* and *in vivo* condition is widely acknowledged by several authors (Chakravarti and Rangarajan, 1966; Rangarajan and Chakravarti, 1969; Thind and Payak, 1972; Saxena and Lal, 1972, 1973, 1974; Randhawa, 1977; Randhawa and Thind, 1978; Randhawa *et al.* 1979; Sinha and Prasad, 1977). Sabet (1956) tried streptomycin (dihydro streptomycin sulphate) and terramycin (terramycin hydrochloride) singly and in combination on *E. chrysanthemi* pv. *zeae* under both condition *(in vitro* and *in vivo).* Both the antibiotics were effective singly and in combination against the bacterium by paper-disc methods. Sinha and Prasad (1977) screened 35 chemicals and 15 were found to be effective in disease control, when applied immediately after the inoculation of plants.

Chakravarti and Rangarajan (1966) studied effect of streptocycline on 16 species of plant pathogenic bacteria. The antibiotic was effective at all the concentrations (25, 50, 100, 250, 500 and 1000 ppm) against *Erwinia* species but *E. chrysanthemi* pv. *zeae* was highly sensitive. Rangarajan and Chakravarti (1969) made another effort and evaluated various antibiotics and fungicides against *Pseudomonas lapsa* and *Erwinia chrysanthemi* pv. *zeae* by paper disc method. Antibiotics namely streptomycin, terramycin and streptocycline were found to be very effective against both the organisms at 100 ppm, while penicillin G was totally ineffective at all the concentration tested. Fungicides like dithane M-22, captan, flytolan, ferbam, bisdithane showed little effect against both pathogens. Many others authors also studied the effect of antibiotics on growth of *E. chrysanthemi* pv. *zeae* (Rangarajan and Chakravarti 1970a; Thind and Payak 1972; Alberghina 1974; Thind and Soni, 1983). In recently, Kumar *et al.* (2016) studied the copper fungicides with combination antibiotics significantly inhibit the growth of pathogen under both conditions (*in vitro* and *in vivo*). Many authors also studied the significance role of alone antibiotics and combination with copper fungicides to control plant pathogenic bacteria in different crops (Raju *et al.* 2011; Ravi kumar *et al*., 2011; Lokesh *et al.,* 2013).

E. chrysanthemi pv. *zeae* is highly sensitive to chlorine. Chlorine has property to completely inhibit the growth of the pathogen at 1 μg/ml under *in vitro* condition. Different techniques of bleaching powder were used such as sprinkling of chlorinated water between plant rows or on basal internodes of plants or broadcasting of dust or granules (coated and uncoated; containing 22 and 28% chlorine, respectively) between thé rows were effective to reducing the disease incidence significantly but the differences among them were not significant. While, application of granules between rows, first at pre-flowering and then 10 days after, was better than the other methods. Drencing of bleaching powder solution (contains 33% of chlorine) containing 100 μg/ml chlorine during 24 hs before, after and at inoculation time reduced the incidence by 70, 20 and 40%, respectively in potted maize plant. Thind and Payak (1972) studied that chlorinated water (100 μg/ml chlorine) reduced the incidence up to 75-92% when drenching applied from knee high stage to flowering stage with 15 days intervals. Similarly, Sharma *et al.* (1982) found that two applications of Klorocin (contains 22% chlorine) at the rate of 250 μg /ml chlorine resulted in significant disease control (48-28%). It was also observed that broad cast of bleaching power in the maize field found effective which acknowledged is widely. Lal and Saxena (1978) applied bleaching powder (25 kg/ha) at two stages, first at flowering stage and the second 10 days after, found significantly result for controlling the disease. Many authors also widely acknowledged the effect of bleaching powder to control bacterial pathogens in different crops (Padmanabhan and Jain 1966; Segall 1968; Lal *et al.* 1970; Dueck 1974; Verma and Upadhya 1974; Thind and Soni 1983; Shekhawat *et al*. 1990; Ghosh and Mandal 2009; Sharma and Kumar 2009). Recently Kumar *et al.* (2016) studied efficacy of five antibacterial chemicals *viz.,* stable bleaching powder, streptocycline, cristocycline, blitox and kocide against *D. zeae*

under *in vitro* and *in vivo* condition. Stable bleaching powder (100 ppm) found most effective to inhibited growth of the pathogen with increased in yielding of three maize cultivars *viz*. Dekalb Double (52.4%), Punjab Sweet Corn-1 (64 %) and PMH-1(57.9%) cultivars.

Biological control: Only few studied available on control of *D. zeae* by biological agents in case of maize as compared to other crops such as potato and tomato. Kumar *et al.* (2016) studied efficacy of bio-agent (*Pseudomonas fluorescence*) against *D. zeae* under *in vitro* and *in vivo* condition. It was observed that *P. fluorescence* found effective only *in vitro* condition not in the field. Kloepper (1983) studied that application of plant growth-promoting rhizobacteria (PGPR) to potato seed, resulted in significant reduction of populations of *E. carotovora* in field trials. Nagaraj *et al* (2012) studied that tip-over disease of banana caused by *Erwinia carotovora* subsp. *carotovora* and *Erwinia chrysanthemi* can be controlled by antagonistic bacteria *viz*., *Bacillus subtilis, Pseudomonas fluorescens* and VAM fungi (*Glomus fasciculatum*).

Conclusion

The *D.* zeae prefer infection in presence of required moisture therefore bacterial stalk rot disease occurring in *kharif* sown maize in India. The *D.* zeae is used pectolytic enzymes as virulent factor due to this its have multi host range. The bacterium survives in soil and host debris, multi host range also help to the bacterium for long survival. The pathogen is characterized by biochemical and molecular tactics. In present, *Pel* gene and rDNA specific primers are frequently used for *D. zeae* characterization. The disease controls in the field condition with help of drenching of 100 ppm bleaching powder and via spray of antibiotics. However, we have more need of work on resistance germplasm and other chemicals to control this disease.

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