PATOGENICIDAD BACTERIANA EN MAÍZ (Zea mays)

Bacterial Pathogenicity IN CORN

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Resumen

El maíz es un grano de consumo mundial para alimento tanto humano como animal. México se encuentra en el séptimo lugar de la producción, China y Estados Unidos son los principales consumidores. Zea mays pertenece a la familia de las gramíneas y tiene producción anual. La planta se puede ver afectada por plagas principalmente de diferentes especies de insectos. Puede también desarrollar enfermedad debida a hongos y virus, así como a bacterias patogénicas. Dentro de estas la causada por organismos del género Pantoea. P. stewartii se sabe que tiene genes como cps que codifican para la producción de exopolisacarido sterwatan controlada por un mecanismo de quórum sensing, y hrp para el sistema de secreción tipo III involucrados en la patogenicidad de la bacteria. En la descripción del genoma de P. ananatis se identifica posibles determinantes de patogenicidad

como para el EPS ananatan; no contiene sistemas de secreción II y III pero si el IV, los cuales pueden estar relacionados con su patogenicidad. Hemos aislado P. ananatis de cultivos de maíz y amplificado secuencias para cps y hrp. Se ha probado la patogenicidad en plántulas de maíz y frijol observándose las lesiones de mancha blanca, clorosis y zonas necróticas de las hojas.

Palabras clave: Zea mays, patogenicidad, Pantoea.

ABSTRACT

Corn is a grain world for both human consumption and animal feed. Mexico is in the seventh place of production, China and the U.S. are the main consumers. Zea mays belongs to the grass family and has annual production. The plant can be affected by pests mainly of different species of insects. It can also develop due to disease fungi and viruses, as well as pathogenic bacteria. Within these caused by organisms of the genus Pantoea. P. stewartii is known to have such cps genes encoding exopolysaccharide production sterwatan controlled by quorum sensing mechanism, and hrp for type III secretion system involved in pathogenicity of the bacteria. In the description of the genome of P. ananatis possible pathogenicity determinants to identify the EPS ananatan; contains no secretion systems II and III but the IV, which may be linked to pathogenicity. We have isolated P. ananatis of corn and amplified sequences and hrp cps. Have been tested for pathogenicity in maize and bean seedlings observed white spot lesions, chlorosis and necrotic areas of leaves.

Key words: Zea mays, pathogenicity, Pantoea.

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Introduction

Corn is a very remote farming, suggesting About Which dates from 7000 years,, Although its, origin is unclear. The oldest findings of plant esta Have Been Found in the highlands of Mexico, so refer to this region as the point of irradiation. This cereal was one of the main products of generations of Indigenous subsistence. Today STI is Widespread cultivation in

all the Other Countries, Especially in Europe and the US Where it Occupies a strategic position (Cooperativa Colonias Unidas. Agropec. Inc. LTDA. Itapúa, 2005, Salvador, 1997).

Among the products That Can Be Obtained from corn are the following:

Protein and fiber to preparing balanced meals.

Sugars: such as dextrose for snacks, bakery products, beverages, will, lysine, citric acid and antibiotics; High fructose corn syrup: as a sweetener for the production of soft drinks, juices, jams, sweets, desserts, wines and low-calorie sweeteners; and Glucose: for the manufacture of sweets, candy and gum.

Ethanol: industrial alcohol, alcoholic beverages and fuel.

Oils: Edible household and baby food.

Starch: to make bread, porridge, baby food, beer, corrugated cardboard and paper.

Coloring: Processes for the production of soft drinks, beer, spirits, meats and bakery.

Maltodextrins: milk, sausage, chocolate powder, powdered food.

Sorbitol: for toothpastes and confectionery (SE, 2012).

In general context it is Known That bacteria Associated with plants can act as beneficial or harmful. All vegetables are Either surface microbiota (epiphytes) or inside (endophytes). Some are residents and other transients. Among the microorganisms are bacteria colonizing plants successively, as mature These Large Populations Become visible aggregates of bacteria in biofilms as well as plugging the vessels of plants. Depending on the type bacterial Populations of approximately 106 CFU / ml (colony-forming units / milliliter) or higher are required for bacteria to function as Either as biological control agents, beneficial purposes or as pathogens Causing Infectious Diseases (Vidaver et al., 2004).

General Characteristics

The corn plant (Zea mays) belongs to the grass family, it is robust and easy development bearing of annual production. The stem is simple, erect, high length (reaching up to 4m high) and without ramifications. By the look reminiscent of a reed, he presents entrenudos and spongy bone. Maize is monoecious inflorescence, with separate male and female inflorescences within the same plant. The male has a panicle inflorescence (called breakwater or plume) of yellowing which has a high amount of pollen in the order of 20 to 25 million grains. In each little flower panicle composing three stamens are presented. The leaves are long, large, very sharp and sharp, lanceolate, alternate, paralelinervas ends, hugging the stem and show the presence of villi in the beam. The roots are fasciculadas and sometimes protrude knots at ground level in those secondary or adventitious roots (Cooperativa Colonias Unidas. Agropec. LTDA Inc., 2006).

Corn is used in both human food and animal products can be obtained from many different botanical varieties grown (OIEDRUS, 2007); among the most important are the following:

a) Zea mays L. var. indentata (. Sturtev) LH Bailey: botanical variety is the most cultivated in the world; It is commonly known as dent corn (dent corn).

b) Zea mays L. var. indurata (Sturtev.) LH Bailey: the corn belonging to the botanical variety, are commonly known by the name of Crystal corn (flint corn). Its grains are horny and hard, glassy and slightly rounded or sharp. The color of the grains is typically orange.

c) Zea mays L. var. saccharata (Sturtev.) LH Bailey: the corn belonging to the botanical variety, are commonly known as sweet corn (sweet corn).

Corn seed is contained within a fruit called caryopsis; the outer layer that surrounds this corresponds to fruit pericarp structure is positioned on the seed coat. The latter is internally composed of the endosperm and the embryo, which in turn consists of the coleorhiza the radicle, plumule or embryonic leaves, scutellum and coleoptile or cotyledon (OIEDRUS, 2007).

The average composition of a corn cariópside belonging to the species Zea mays L. var. indentata (. Sturtev) LH Bailey, is 65 to 70% starch, 1 to 2% sugar, 10 to 11% protein; by 4

to 5% ce fiber half (2 to 2.5%) of fiber and 1 to 2% ash. The percentage of relative humidity is between 12 and 13% (OIEDRUS, 2007; FAO, 2007).

Corn production

The level of maize production depends on the area under the crop, and yields it. The Department of Agriculture (USDA) estimates that the 2012/13 world corn production will be 839.68 million tons and 40.7 million lower than harvested during the years 2011 and 2012 (Agropanorama, 2013 tonnes ; FAO, 2007; SE 2012).

The main producing countries of corn a year are:

United States with 272.4 million tons

China produced 200.00 million tons

Brazil 70.0 million tons

European Union (27 States) 54.64 million tons

Argentina 28.0 million tons

Ukraine 21.0 million tons

Mexico 20.7 million tonnes

(Agropanorama, 2013).

United States is the largest consumer of corn 261.67 million tons representing 33.9% of world consumption, China second with 19.29%, the European Union in third with 8.22% and Mexico is fourth with 4.14% (SIAP, 2012).

The market for domestic corn is composed of several varieties among which are white and yellow maize, but there are other varieties like corn color and pozole, with the first two varieties are occupying an important role in the production and marketing. (SE, 2012).

Production in Mexico for the period 2012/2013 is mainly corn grain 17,635,417.30 Tons, with a yield of 7.28 tons / ha; followed by 9,605,147.84 Tons forage maize with a yield of 30.86 tons / ha; followed by grain and seed corn for popcorn with a production of 24,721.94 and 472.60 respectively Tons (SIAP, 2013).

Mexico is a country historically deficient in the production-consumption of corn because domestic production is not enough to supply the required demand. The import market, collection and marketing is concentrated in few companies who store and distribute it, not allowing the entry of new competitors (SE, 2012).

Pathologies caused in maize

Corn, like many other crops are susceptible to various diseases that affect the development of the plant and its fruit. The diseases are favored by environmental conditions, soil type, the susceptibility of the material, and when they are diseases caused by viral agents, conditions that favor the migration, survival and development of vectors (Male and Sarria, 2007 is affected).

The main maize diseases are usually caused by fungi and viruses, causing great economic losses, less disease caused by bacteria that cause relatively minor damage and economic costs are presented. Most plants, wild and cultivated have innate immunity or resistance to many pathogens. However, many plants can harbor pathogens without developing symptoms (asymptomatic) (Vidaver et al, 2004; Infoagro, 2013).

There are different pathologies in corn, some are due to the presence and establishment of pests such as insects, they wireworm that occurs in sandy soils rich in organic matter. These worms are beetles of the genus Conoderus and Melanotus, causing serious deterioration on the ground and even death. The gray worms are larvae of moths of the genus Agrotis class. Ípsilon Agrotis species causes damage to the neck of the plant producing them serious injuries (Infoagro, 2013).

As Rhopalosiphum padi aphids feed on the sap causing a decrease in the final yield. While green corn aphid is Rhopalosiphum maidis virus transmitter to extract the sap from plants mainly attacking the sweet corn, the latter species also causes serious damage due to rapid growth of corn. The European corn borer Ostrinia nubilalis, a stem borer and develops 2-3 generations reaching their full larval development reaching 2 cm in length. The larvae begin feeding on corn leaves and eventually introduced into the stem. Just breaking the stems and cobs that were also damaged (Infoagro, 2013).

Corn borers are two plagues caused by Sesamia nonagrioide, a lepidopteran whose drill caterpillar corn stalks causing extensive damage. The caterpillar measures about 4 cm, overwinters inside cornstalks where they form pupae. Butterflies appear in spring depositing eggs on the leaf sheaths; Pyrausta nubilalis, this lepidopteran caterpillar measuring about 2 cm in length, which damage occurs by eating leaves and cornstalks dig. Egg laying takes place in different parts of the plant (Infoagro, 2013).

Some examples of major bacterial disease caused by Pseudomonas we alboprecipitans, it manifests itself as spots on the leaves white with reddish causing stem rot. H. turcicum affects the lower leaves of the corn. The spots are large from 3 to 15 cm and the blade is turning from green to brown. Their attacks are more intense at temperatures of 18 to 25C. Leaves fall if the attack is very marked. The Antranocsis is due to the presence of Colletotrichum graminocolum. Injuries are causing reddish-brown spots and are located in the leaves, limbo produce wrinkling and destruction of the sheet (Infoagro, 2013; Male and Sarria, 2007).

Bacterial plague Acidovorax avenae subsp. avenae, causes stripes or spots long, narrow leaf with brown edges, the leaves are cut and this can easily be associated with higher stalk rot. Burkholderia andropogonis produces long, parallel stripes of olive green to yellow and moisture damage (soaked), the upper leaves may be almost white. Wilting of the Northern corn leaf Setosphaeria turcica produced results similar to forms long axis gray-green spots; but wilt leaf produced by Cochiobolus heterostrophus, and leaf spot of corn produced by C. carbonum cause distinct orange to brown spots. (Stack et al, 2002).

Clavibacter michiganensis sbsp. nebrakensis causes symptoms similar to Stewart's disease, with lesions parallel to the veins of the leaves of green intense black wet look. In infection Pseudomonas syringae pv. syringae, small patches appear on the tips of the lower leaves of green that later turns to reddish brown. In advanced lesions yellowish halo is observed around them. Xhantomonas translucens is transmitted bacterial testing seeds and bay Rathayibacter tritici attacks with rot pin (CIMMYT, 2013).

Pantoea stewartii causes Stewart's disease reported in 1897 in New York on sweet corn. Currently it does not appear significantly in Central and South where corn originated America. Pantoea stewartii subsp. stewartii can also be transmitted from soil, manure or corn stalks during the cold winter. The main agent responsible for the dispersion in USA is the beetle Chaentocnema pulicaria, but also can occur in other vectors such as Diabrotica undecempunctata howardii (both adult and larvae) Chaentocnema denticulata, the larvae of Delia platura, Agriotes mancus, Phyllophaga sp . and the larvae of Diabrotica longicornis. When the beetle migrates, can be brought from considerable distances by air currents and transmit the bacteria (OEPP / EPPO, 2006).

The main host is sweet corn (Zea mays var. Saccharata) the most susceptible, but the teeth (Z. mays var. Indentata) which is usually tougher, flour and corn for popcorn cultivars (Z. mays var . everta), sorghum and millet (Margaret et al, 2004). P. stewartii was artificially inoculated lachrymajobi Coix, Zea perennis Setariapumila and causing damage to the plant (OEPP / EPPO, 2006, Stack et al., 2002, Merighi M., 2003).

The bacterium also attacks other cultivated forage grasses in North America as Tripsicum dactiloydes and teosinte (Zea mexicana). Notable exceptions have been reported sporadic outbreaks reported sweetcorn in Italy, Austria and Mexico (Toluca Valley, Oaxaca, Tabasco, Tlaxcala and Veracruz). In these countries it is unknown how the disease spreads through an initial focus and the subsequent establishment, but in countries where if present, may show the potential capacity to accommodate P. stewartii vector combinations or the presence of host side (Stack et al, 2002).

Symptoms of striped sheet can be confused with nutritional deficiencies or drought. The presence of Pantoea ananatis as causing white spot, chlorosis and necrosis with crop losses (Pérez-and-Lump et al, 2009) are also reported.

Pathogenicity

Two groups of genes that play a role in the pathogenicity and virulence of bacteria infecting maize. In this chapter reference is made to the genus Pantoea. One is that of cps comprising 12 genes and is required for the production of exopolysaccharide (EPS) "sterwatan", the other is the group hrp, encoding a type III system secretion, necessary for general pathogenicity and production from leaf lesions (Cha et al, 1998).

The cps cluster, specifically in the region of cpsD and CPSE encodes proteins responsible for synthesis and secretion assembly of repeating units and its polymerization within a macromolecule exopolysaccharide (EPS) known as sterwatan. This EPS blocks the free flow of water, leading to wilt condition. It has been proposed that the EPS can work to prolong Wts symptoms (symptom soaking water) and promote bacterial growth to hold water and nutrients in the intercellular spaces (Coplin et al, 1992).

The sterwatan is a unit of approximately 45MDa, repeated seven monosaccharides containing glucose, galactose and glucuronic acid in a 4: 2: 1 provides a protective barrier against the defense factors of the host plant and partly contributes to induction of the early symptoms of the disease (Coplin et al, 1992; Labate et al, 2007; Merigui et al, 2006).

EPS production is controlled by a mechanism of quorum sensing and is regulated by the SSF protein produced by SSF, which is synthase signal homoserine lactone (HSL) and EsaR produced by esaR, which is the transcriptional regulator governing the locus cps (Coplin et al, 1992; Wang and Leadbetter, 2005; González and Keshavan, 2006, Minogue et al, 2005).

If the SSF sequence is mutated, the production of 3-oxohexanoyl-HSL and some other detectable acyl-HSL is blocked; this deficiency suppresses the production of EPS and eliminates the ability of P. stewartii produce Stewart's disease in the host plant. Meanwhile, when a mutation in esaR produces high levels of EPS, regardless of cell density and even in

the absence of HSL signal is given. EsaR EPS suppresses synthesis at low cell density (von Bodman and Farrand, 1995; von Bodman et al, 1998).

To stop the repressed gene requires micromolar amounts of constitutively producing EPS HSL, but strains are less virulent than the wild type. It is suggested that quorum sensing mechanism may take EPS expression during the early stages of infection, making this not interfere with other mechanisms of pathogenesis (von Bodman et al, 1998, Minogue et al, 2005).

EPS is required for formation of biofilm and rapid systemic movement in the plant deficient strains EPS grow and spread more slowly in vascular tissues of the host, are less like the type strains to colonize the plants systemically and they are unable to cause wilt infected plants (Merighi M., 2003).

The synthesis of the capsule is constitutive but additional slime production is induced by the availability of free sugars in the growth medium. To sum all this is involved in virulence, EPS possibly hits a mechanism for protecting fitoaglutininas in the ducts and colonization of the insect vector. Most of these genes that occur are conserved in E. amylovora and E. coli based on sequence homology and interspecies complementation tests, except for some glycosyltransferase genes which may be considered different in the structure of the two polysaccharides (Merighi M, 2003).

The cloned genes ams complemented E. amylovora most cps P. stewartii mutants for production of silt and virulence in seed corn, but the arrangement of the genes in the two clusters showed slightly different (Merighi M. 2003, Sharples et al, 1990).

The hrp genes are dispensable for development in minimal medium, but essential for pathogenicity in susceptible hosts and to cause hypersensitive response (HR) in inconsistent and non-host hosts. The HR is a rapid, localized defense plant involving programmed cell death, production of active oxygen and formation of phenolic compounds and antimicrobial around the infection site. During natural infection, the reaction usually is not visible because they are involved few host and bacterial cells, but under experimental conditions with high inoculum (> 107 / ml), a massive cellular collapse and a confluent necrosis which is seen with the naked cause sight. This reaction has the net effect of restricting the growth

of the pathogen and the subsequent progress of the disease (Merighi M, 2003; Merighi et al, 2001).

The HRP / HRC proteins secreted by the TTS (type III secretion system) systems, have been named Hops (by external protein HRP), parallel to the designation of Yops Yersinia outer proteins. At least four classes of Hops travel the secretion apparatus: Harpins, pilins HRP translocator proteins and effector proteins. The first three are collectively defined as helper proteins proved for their alleged or auxiliary role in translocation / secretion of other substrates of TTS systems (Frederick et al, 2001).

The harpins hydrophilic proteins are rich in glycine and cysteine free heat stable, are able to elicit an HR when infiltrated at high concentration are within the apoplast. They travel through the TTS device but are released within apoplásticos spaces rather than be injected into the host cells. The harpins have been described in E. amylovora (Ea harpin or HrpN and HrpW) erwinias other, in all of P. syringae pathovars and R. solanacearum studied, but there is no sequence similarity between the proteins HrpN, HrpW , hrpZ and PopA1 (Frederick et al, 2001; Merighi et al, 2006).

Each phytopathogenic bacteria respond somewhat differently to external and development conditions, and signals necessary to induce the expression of hrp genes in P. stewartii, Pseudomonas syringae and Erwinia amylovora (Merighi et al, 2001). HRPS regulation is carried out by both specific and global regulators, which can be "tuned" according to the conditions of each niche in which the pathogen (Merighi et al, 2001) is found.

In the species Pantoea ananatis it is has reported the genome sequence in the pathogenic strain of Eucalyptus LMG20103. The genome consists of a single chromosome with a size of 4.69 million nucleotide and G + C content of 53.69% and a total of 4.27 genes coding for proteins. It has revealed the presence of 433 genes encoding proteins which demonstrated experimentally to play a role in the disease (Maayer et al, 2010).

Lack of secretion systems types II and III also reported, which play an important role in disease pathogenic bacteria both animals and plants; but three copies of type IV secretion system, recently described as pathogenicity factor and where the putative secreted effector protein secretion via these systems, is acquired through horizontal transfer. Some possible

pathogenicity determinants as exopolysaccharide ananatan demonstrating that plays a role in the disease of onion and pineapple (Maayer et al, 2010) were identified. The sequence of the P. ananatis strain PA13 comprises pseudogenes 4.87 Mb is 55 7 83 rRNA operons and tRNAs and 281.754 bp plasmid (Choi et al, 2012).

We reported earlier identification by 16S rDNA of P. ananatis isolated corn plants with severe damage (Pérez-and-Lump et al, 2009). Samples were obtained from leaves, stem and soil attached to the roots of corn plants of the states of Puebla, Tlaxcala and Veracruz in Mexico, identifying 32 strains.

Furthermore, we have used oligonucleotide primers CPSL1 / CPSR2c described by Coplin et al in 2002 to 50% amplifying strains identified by PCR the fragment of 1100 bp described. These oligonucleotides are targeted to genes cps cluster, specifically in the region of cpsD and CPSE coding for the synthesis of proteins responsible for assembly and secretion of repeating units and its polymerization within a macromolecule exopolysaccharide (EPS) known as sterwatan.

Identification and pathogenicity tests

The use of HRP1d / HRP3c designed to amplify a 900 bp fragment frame open reading HRPS gene oligonucleotides, we reported 37.5% of the strains with the fragment, and some of which were also amplified with oligonucleotides cps. The HRPS gene, using the first hrp1d and hrp2c, are internal to the open reading frame for HRPS reading. The amplification product thus obtained was 900 bp, encoding a transcriptional enhancer NtrC similar to that required for the expression of hrp secretion genes (hypersensitive response and pathogenicity) and the effector wts (Coplin et al, 2002). Strains that showed the amplified fragment (55.5%) were positive hypersensitivity response in snuff.

The use of ES1G1 / ES1G2c and ES1G2c / ES16 oligonucleotides amplified by PCR, fragments of 920 and 290 bp respectively in all strains of P. stewartii (Coplin et al, 2002). Also shown in our tests (16.6%), we both amplifications, but (62.5%) only show the amplification fragment of 920 bp, this would be inconsistent with the report that the

chromosomal regions encoding ITS rRNA vary considerably related species (Coplin et al, 2002). However, they also report that P. ananatis also amplifies the 290 bp fragment and other nonspecific bands for both sets of oligonucleotides.

When performing the inoculation of maize seedlings 17 days of growth, to determine pathogenicity strains identified as Pantoea used. All these strains were able to cause a visible when inoculated seedlings at a dose of 1X106 CFU in developing corn plants damage. It was drastically reduced the size of the taproot and the size and number of secondary roots, showing lack thereof in some plants. The stems were also reduced in size and leaves also some plants that lacked both. It was observed that further deterioration plants also showed increased turbidity in the culture medium, suggesting an increase in bacterial population.

In performing these tests with five of our strains identified as P. ananatis in Phaseolus vulgaris bean seedlings (replicas of 5) we obtained similar to those presented in corn, where the strains caused total damage to seedlings results.

Conclusions

Search Pantoea species of soil, stalks and leaves of corn crops visually impaired in the states of Puebla, Tlaxcala and Veracruz in Mexico allowed us to detect the presence of Pantoea ananatis.

The pathogenicity test corn seedlings showed severe damage to strains isolated from three different plots.

was determined and identified a putative partial sequence for the locus of P. ananatis cpsD.

HRPS genes for amplified.

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