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INCIDENCE OF *PANTOEA AGGLOMERANS* ASSOCIATED WITH BARNYARD GRASS (*ECHINOCHLOA CRUSGALLI* L.) IN PAKISTAN

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ABSTRACT

Weeds are reservoirs for the survival of pathogenic microorganisms and cause of spread of infectious disease of plants through vectors. To minimize the spread of bacteria to other host crops, proper characterization is required to suggest suitable control strategy. Survey was conducted in December 2015, leaf blight was observed on barnyard grass growing in surroundings of citrus orchards located in Lahore, Pakistan. Isolation of the bacterium on nutrient agar medium was performed and their characterization based on morphological and biochemical tests. 16S rRNA was amplified directly with primers 27F/1492R, cloned and sequenced. Sequences were blast in NCBI data base which showed 99% identity to *P. agglomerans* strain. A neighbor-joining tree was generated with reported bacterial strain that were most closely isolated strain *P. agglomerans* (LT592133). Pathogenicity test was conducted on barnyard grass with bacterial suspensions having 1.5×10^8 CFU/ml in controlled conditions to confirm Koch's postulates. According to results this is first report on the incidence of *Pantoea agglomerans* on Barnyard grass in Pakistan.

Keywords: *Pantoea agglomerans*, pathogenicity test, barnyard grass, 16S rRNA analysis

INTRODUCTION

Barnyard grass is a species of grass that is included in the Global Compendium of Weeds as well as among the world's worst weeds (Pheng *et al.*, 2001). It has been stated as weed in many crops throughout tropical and temperate regions of the world. It is also considered an environmental weed that has become invasive in natural grasslands, coastal forests and spreads in Asia, Africa, Australia, Europe and America. Weeds are reservoir for majority of pathogens including bacteria that leads to the emergence of new strains of bacteria that may infect crops once a susceptible host plant is present into an agro-ecological system. Bacterial population from weeds should be identified to suggest some control measure,

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against the spread of pathogenic bacterial disease to economical important crops.

Pantoea agglomerans is a gram-negative aerobic, rod shape bacteria belongs to family Enterobacteriaceae. All species of the genus *Pantoea* can be isolated from different plants and soil (Andersson *et al.*, 1999), where they can be either pathogens or nonpathogenic (Monier *et al.*, 2005). *Pantoea agglomerans* cause leaf blight symptoms as necrotic lesion that appeared as water soaked or light brown to slightly reddish spots on the upper blades of the leaves, which ultimately turned into blight and then stalk rot (Morales *et al.*, 2017). This species of *Pantoea* is infectious to human which is associated with different plants. Though, this bacterium could be a reason for the spread of opportunistic infections in human with plant materials. It can be epiphyte or endophytic on different plants mostly occurs with mutualistic relationships with plants. However, this species has also been identified as a major cause of diseases in a variety of hosts such as rice, maize, sorghum, onion, cotton, walnut, bamboo. Almost several

of plant-pathogenic strains of *P. agglomerans* can cause tumors, which includes gall formation on table beet, an ornamental plant gypsophila, Douglas-fir, cranberry and wisteria. Currently, the reported specie has been recognized as a cause of bacterial blight disease in the edible mushroom which is cultivated in China. Various factor are involved in the mechanism of pathogenicity among strains o *Pantoea agglomerans* which involves the hypersensitive response, quorum sensing, phytohormones and the type three secretion system through which it inject the effector proteins into the cytosol of a plant cell and become the cause of infection in plants (Dutkiewicz *et al.*, 2016).

MATERIAL AND METHODS

In December 2015, leaf blight was observed on barnyard grass (*Echinochloa crus-galli*) growing in surroundings of citrus orchards located in Lahore, Pakistan. Symptomatic leaf tissue sections of about 1 cm² was disinfested with 1% sodium hypochlorite for two minutes followed by three subsequent washings with sterile distilled water, grinded in distilled water by using sterilized pestle and mortar. Then it was streaked on nutrient Agar medium and incubated at 30 °C for 24-48 Hours. After 3-4 days, bacterial colonies were observed and preceded for morphological and biochemical characterization. This strain was identified through 16S rRNA gene sequence analyses. 16S rRNA was amplified directly with primers 27F (AGAGTTTGATCMTGGCT CAG)/ 1492R (ACCTTGTTA CGACTT) cloned and sequenced (Macrogen Korea). Pathogenicity tests were performed to confirm Koch's postulates. Phylogenetic analysis were performed using Mega 6.0 software.

RESULTS AND DISCUSSION

Owing to the significance of weed hosts in the establishments of new bacteria strain, Barnyard grass display necrotic symptoms on leaves were evaluated from Lahore in December 2015. Isolated bacterial strain was preceded for morphological characterization. Morphologically Colony was off white, round, smooth and shiny with wavy margins, concave from elevations on nutrient agar medium. This bacterium was facultative, anaerobic, gram-negative, rods with three to six peritrichous flagella, 1.0 to 3.0 × 0.5 to 1.0 µm in size. Strain was negative for spore type, gram type, capsule stain, indole and methyl red test,

hydrogen sulphide, oxidase test, growth at 2% NaCl, growth at 25 and 40°C, Arginine dihydrolase, sorbitol fermentation, rhamnose fermentation but positive for motility, citrate utilization, nitrate reduction, catalase tests. Representative isolate was selected for pathogenicity tests and subsequent 16S rRNA analysis. Pathogenicity tests were conducted on disease-free citrus plants (having five to six leaves) tomato, common sow, lambs quarters, and mallow weeds. Biochemical tests confirm the presence of *Pantoea agglomerans*. DNA was isolated from bacterial culture by CTAB method (Wilson 2001). 16S rRNA was amplified directly with primers 27F/1492R, cloned and sequenced (Accession No. LT592133). BLAST of 16S rDNA sequence showed 99% identity to *P. agglomerans* strain type 48b/90 (FJ756354) (Volksch *et al.*, 2009). A tree was constructed for the isolated clone using Mega 6.0 with the reported bacterial strains using neighbor-joining method. *P. agglomerans* (DQ307453) were most closely related to *P. agglomerans* (Gene Bank LT592133) with an average 16S rRNA sequence similarity of 99 %. Phylogenetic analysis grouped *P. ananatis*, *P. stewartii*, and *P. agglomerans*, *P. brenneri* into three clusters (Figure 1). Our sequence was clustered with group of *P. agglomerans* strains. Pathogenicity of the isolate was confirmed by injecting (10⁸ CFU mL⁻¹) of inoculum into 3 months old seedling stem of citrus in the greenhouse at 28 C and 80% relative humidity and 12 h photoperiod were maintained. Inoculations with sterilized water served as controls and all treatments were replicated thrice. Water-soaked necrotic lesions on young leaves after 8 days post inoculation were observed, while negative control seedlings did not displayed any symptoms. Koch's postulates were confirmed through reisolation of culture, and characterize based on morphology and biochemical tests. The re isolated culture were 100% similar to their original isolate. *P. agglomerans* has been reported to affect other host crops, comprising, pearl millet in Zimbabwe (1997), onion in the South Africa (1981) and USA (2006), maize and Sorghum in Mexico (2007), Chinese taro in Brazil (2007), *V. angularis* in china (2015), onion in the United States (Tho *et al.*, 2015) and cotton in China (Ren *et al.*, 2008). *P. agglomerans* also found widely in nature on leaves, fruits, and the seeds of many crops and is a known endophyte (Feng *et al.*, 2006).

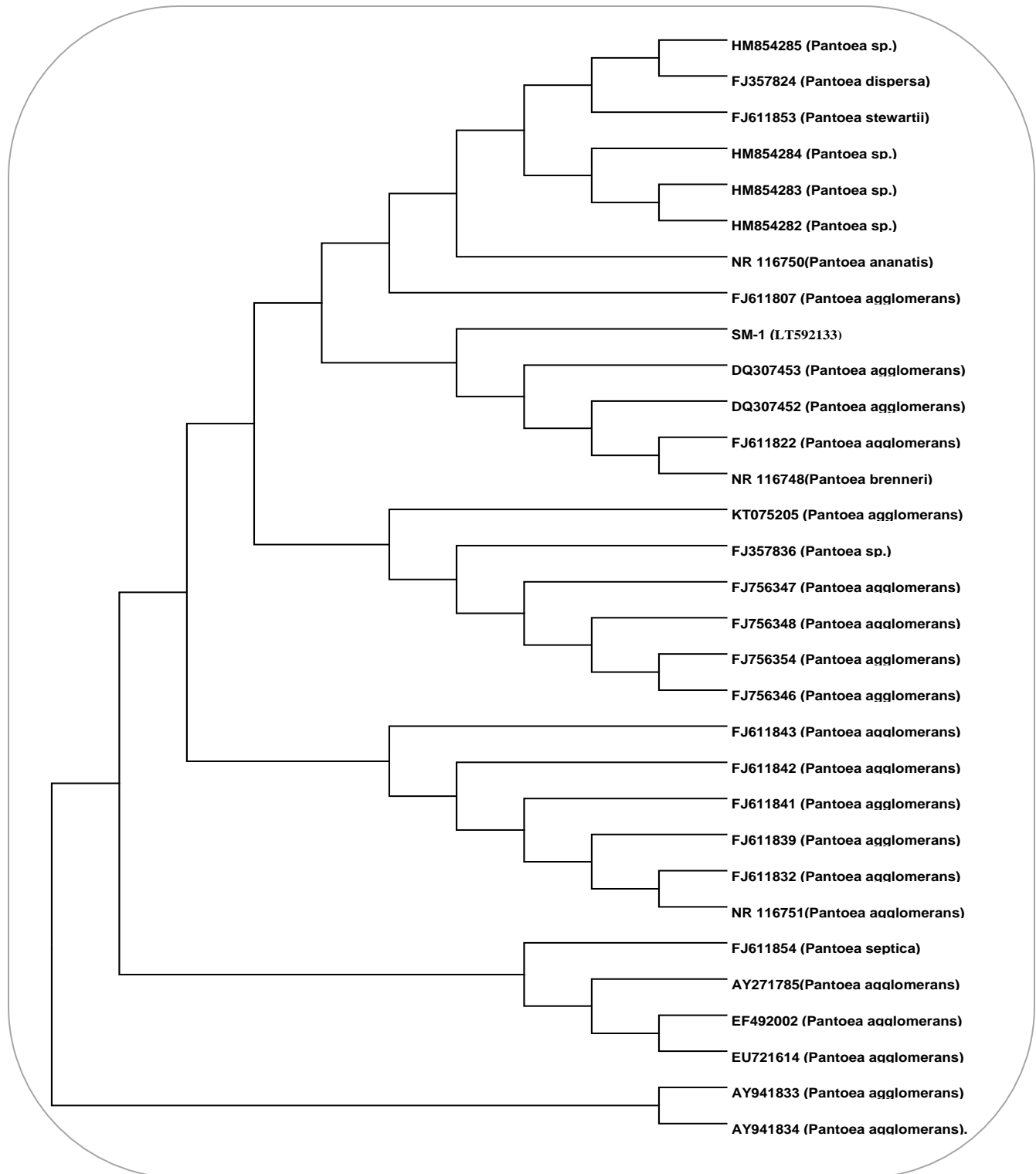


Figure 1. Phylogenetic tree constructed with sequences of the 16S rRNA regions of *Pantoea agglomerans* bacteria isolated from barnyard grass and sequences from GenBank (indicated by accession number), using the neighbor-joining method.

However, to our understanding, this is the first report on the incidence of *P. agglomerans* associated with leaf of barnyard grass in Lahore Pakistan. We isolated this

pathogen strain of bacterium from grass that could be a source of infection for other economically important crops. Management of this grass type weed should be

planned in order to limit the spread of this bacterium to other hosts.

This bacterium culture was submitted to First fungal culture bank of Pakistan with accession 637.

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