PSEUDOMONAS FLUORESCENS – ОСНОВЕН И СЪПЪТСТВАЩ ПАТОГЕН ПО РАСТЕНИЯ, ФОРМИРАЩИ ЛУКОВИЦИ

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PSEUDOMONAS FLUORESCENS – A PRIMARY AND SECONDARY PATHOGEN OF BULBOUS PLANTS

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ABSTRACT

Pseudomonas fluorescens is a widespread species which survive in soil and water with different chemical composition and different climatic conditions. It is a secondary pathogen in nosocomial infections in hospitals because of its strong variability. In phytopathology it is known as a part of the soil resident microflora and as a co-pathogen in various plant diseases. In the recent years these bacteria are more often isolated together with other phytopathogenic bacteria, but also alone from diseased bulbs of various plants. This study proves the pathogenic potential of *Pseudomonas fluorescens* isolates as a primary or secondary causal agent of diseases in plants.

***Key words**: Pseudomonas fluorescens, phytopathogen, bulbs*

INTRODUCTION

P. fluorescens is known mainly as a saprophyte but it is also associated with the spoilage of food: eggs, meat, fish, and milk (Palleroni, 1984). *P. fluorescens* is a heterogeneous species that is now divided into five biovars (Palleroni, 1984). Biovar V is most various in nutritional needs of the strains and includes strains which have lost some diagnostic characteristics (Palleroni, 1984;Bradbury, 1986;). The phytopathogenic strains cause soft rot in onions, garlic, hyacinth, gloxinia, dahlia, lettuce, cabbage, alfalfa, flax, tobacco, potato and some less-known plants in Europe and Russia (Kabashna, 1975; Bradbury, 1986). An atypical strain is a pathogen of six genera of cacti (Kabashna, 1975; Bradbury, 1986). Some strains induce systemic resistance, a phenomenon in which hypersensitive response in plants is activated instead of disease development, by at least two mechanisms (Nelson, 2004; Compant et al., 2005).

P. fluorescens is a usual inhabitant of soil, natural and waste waters and for the last two decades it has been known as a major nosocomial pathogen with a haemolytic activity (Gibb et al., 1995). However, in 20thC it was generally accepted that phytopathogenic *P. fluorescens* attacked only weak plants or participated in mixed infections with other pathogens as a secondary pathogen (Elloitt, 1951; Palleroni, 1984; Bradbury, 1986).

This study focuses on the role of *P. fluorescens* in pathogenic processes in different plant hosts.

MATHERIALS AND METHODS

Plant samples. Bacterial strains were isolated in the period 2006-2014 from diseased leaves of snowdrop and summer snowflake and bulbs of onion, garlic, and wild garlic. Isolations were made according to the standard procedure (Rudolph et al., 1990). Single strains were obtained as distinct colonies on King's medium B after cultivation at 28 °C for 48 h and subjected to subsequent purification procedures. Nutrient Agar was used for preservation at 4^{o}C.

Pathogenicity. The pathogenic potential of the bacteria was examined by the hypersensitive reaction (HR) test (Klement, 1963) and artificial inoculations of bulb scales of summer snowflake

and onion (Stoyanova et al., 2005). Pathogenicity tests on leaves of summer snowflake were held with the vacuum infiltration method (Bogatzevska and Kondakova, 1992). Bacterial suspensions of 10^7 cfu/ml grown on Potato-Dextrose Agar medium at 28°C, for 24 h were used.

The symptoms of disease (visual change of color, consistency, odor, and turgor of the tissues compared to controls) were observed periodically between the 1st and 7th day.

Identification and characteristics. The strains were primarily differentiated and characterized following the classic diagnostic scheme described by Schaad (2001). Biolog™ metabolic fingerprints were obtained. Microlog™ 4.20.05 (Biolog™) was used for identification. The data from metabolic profiles was further cluster analyzed through the SPSS procedure by the Ward's method. The matrix of similarity between the isolates was calculated using the Squared Euclidean distance.

RESULTS

Diseased tissues of bulbs were watery, soft, yellow-brown tissues and necrosis of the bulb bottom. The garlic bulbs lacked roots. Symptoms on leaves of snowdrop and summer snowflake were oblong light spots which withered in time (Fig. 1).

Most of the isolated bacterial strains did not induce hypersensitive reaction on tobacco leaves. The HR positive strains were isolated from onion, wild garlic, snowdrop and summer snowflake. Leaves of tested host plants reacted with watery, chlorotic lesions on places of infiltration which expanded, darkened and necrotized until the 5th day after artificial inoculation (Fig. 1).

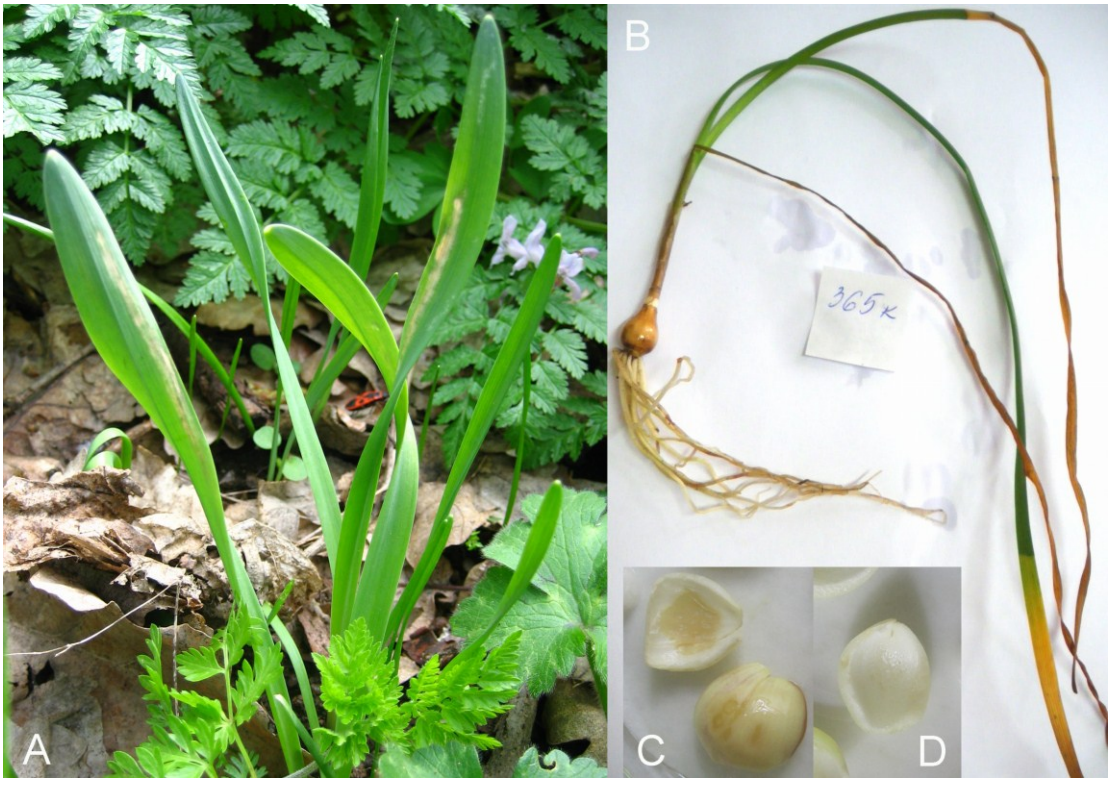

Fig. 1. Symptoms of infection with *P. fluorescens*: A - natural infection of snowdrop; B - artificial inoculation of leaves of summer snowflake; C - artificial inoculation of onion scales; D – onion scale inoculated with distilled water (negative control)

The tested onion scales developed watery slightly yellow colored lesions which darkened with time with tissue collapse (Fig. 1).

The strains were Gram-negative, oxidase-positive aerobs, arginine-dihydrolase positive, synthesizing a fluorescent pigment on King's B medium. All of them were identified with

BIOLOG™ system as *Pseudomonas fluorescens* (with probability 81-100%, distance index 7.96-2.64 and similarity index up to 0.836.

The strains shared their reaction to only 19 of the substrates. They utilized D-galactose, α-D-glucose, cis-aconitic acid, citric acid, L-asparagine, L-aspartic acid, L-glutamic acid, L-proline, L-serine, and D-mannitol and did not use as a sole carbon source α-cyclodextrin, N-acetyl-D-galactosamine, gentibiose, α-D-lactose, lactulose, D-melibiose, β-methyl-D-glucoside, 2,3-butanediol, and D-glucose-1-phosphate. With one exception all the strains do not assimilate also maltose. The strains differed in their reaction to all other tested substrates: dextrin, glycogen, tween 40, tween 80, N-acetyl-D-glucosamine, adonitol, L-arabinose, d-arabotil, cellobiose, i-erythritol, D-fructose, L-fucose, m-inositol, D-mannose, D-psicose, D-raffinose, L-rhamnose, D-sorbitol, sucrose, D-trehalose, turanose, xylitol, methyl pyruvate, mono-methyl succinate, acetic acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α-hydroxybutiric acid, β-hydroxybutiric acid, γ-hydroxybutiric acid, p-hydroxyphenylacetic acid, itaconic acid, α-ketobutyric acid, α-keto glutaric acid, α-keto valeric acid, lactic acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, bromosuccinic acid, succinamic acid, glucuronamid, alaninamide, D-alanine, L-alanine, L-alanyl-glycine, glycyl_L-aspartic acid, glycil-L-glutamic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-pyroglutamic acid, L-serine, L-threonine, carnitine, γ-aminobutyric acid, urocanic acid, inosine, uridine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, glycerol, α-glycerol phosphate, D-glucose-1-phosphate, and D-glucose-6-phosphate.

The cluster analysis divides the strains into two groups (A and B) (Fig. 2).

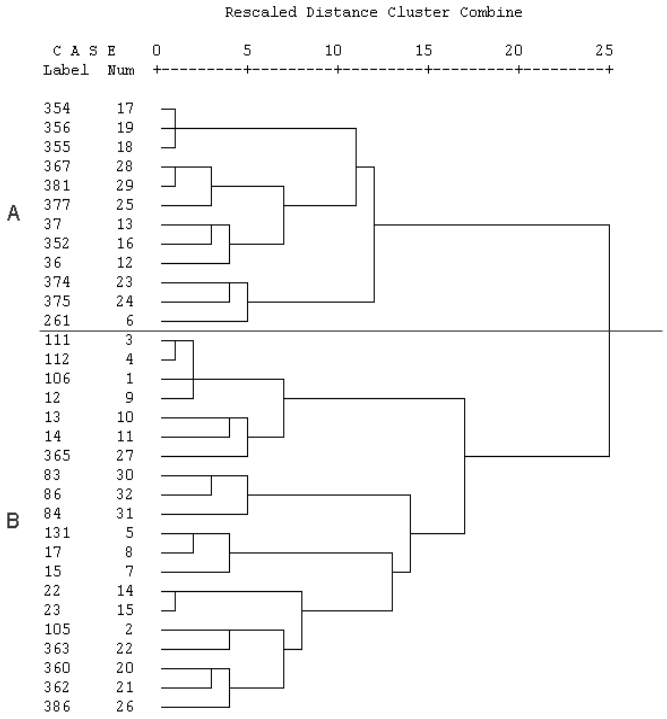

Fig. 2. Cluster analysis of *P. fluorescens* strains according to their Biolog^{TM} metabolic pattern

Cluster A was more homogeneous compared to cluster B and shared reaction to 11 more substrates (positive to adonitol, L-arabinose, D-arabinose, fructose, D-galacturonic acid, D-gluconic acid, propionic acid, succinic acid, and D-alanine and negative to sebacic acid and phenylethylamine). The strains in cluster B shared their reactions to 3 substrates more than the common ones: positive to L-alanyl-glycine and L-pyroglutamic acid and negative to thymidine (Fig. 3). Cluster B comprised isolates from all discussed hosts – onion, garlic, wild garlic, snowdrop and summer snowflake while cluster A included only isolates from onion and snowdrop.

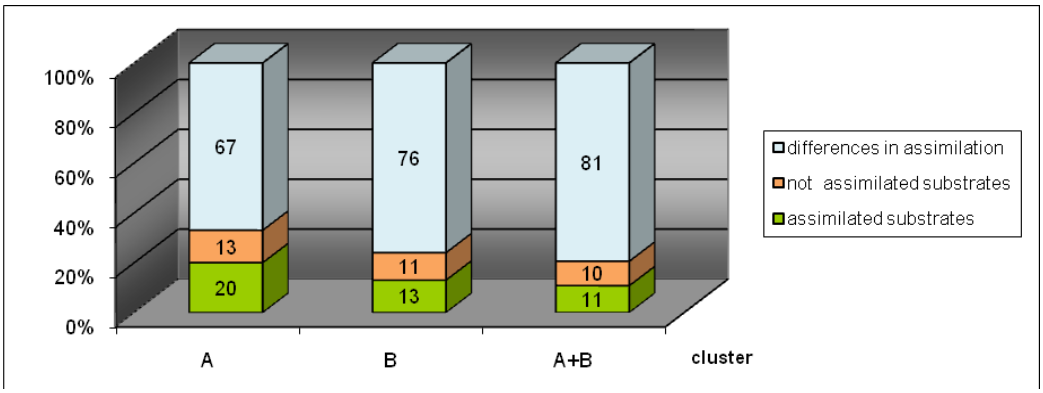

Fig. 3. Assimilation of the substrates included in Biolog GN microplates, according to the cluster in %

DISCUSSION

P. fluorescens was isolated from different plants in a period of several years (2006-2014). Since it has been continuously isolated from onion, garlic, wild garlic, gladiolus and hyppeastrum the beginning of the century (Stoyanova et al., 2005), this species has made its position among the pathogens of bulbous plants. In this study *P. fluorescens* was isolated as a primary (sole) pathogen from most samples of all described hosts – onion, garlic, wild garlic, snowdrop and summer snowflake and in combination with *Burkholderia gladioli* or representatives of *Enterobacteriaceae* family from a few samples from onion and snowdrop. In the cases of mixed infections, symptoms induced from *P. fluorescens* after artificial inoculations of test plants developed slower compared to the isolated co-pathogens and the overall virulence of *P. fluorescens* at artificial inoculations seemed to be lower compared to the pathogenic co-agents. As *P. fluorescens* was mainly described as a weak pathogen the past century (Elloitt, 1951; Palleroni, 1984; Bradbury, 1986), it most probably acted as a secondary pathogen also in these cases of mixed infections. However, based on the data from the last 14 years it can be stated that unlike the past century *P. fluorescens* raises its role in diseases of bulbous plants from an occasional secondary pathogen to a common primary pathogen. To our knowledge snowdrop and summer snowflake are reported as hosts for the first time.

CONCLUSION

P. fluorescens occurs as a primary or a secondary pathogen in leaf or bulb infections of bulbous plants with a tendency to raise its role as a disease agent. The population of the pathogen revealed heterogeneity. This is the first report of snowdrop and summer snowflake as hosts of *P. fluorescens*.

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