PHYTOPATHOGENIC *SERRATIA RUBIDAEA* ISOLATED FROM TULIPS

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ABSTRACT

Serratias are widely distributed in soil, air and water, plant- and animal-associated bacteria. Only *Serratia marcescens* has been established as a phytopathogen. In this study we isolated phytopathogenic *Serratia rubidaea* from imported in Bulgaria diseased Holland tulip bulbs. The strains caused hypersensitive reaction in tobacco leaves and were pathogenic to onion after artificial inoculation. Identification was performed by the BIOLOG™ system. The properties of the strains were shared by their reisolates.

S. rubidaea has been isolated from spoiled coconut and is not known as a phytopathogen. This paper represents the first report of *S. rubidaea* causing bacteriosis of tulip bulbs.

***Key words**: Serratia rubidaea, phytopathogen, tulip, bulb*

INTRODUCTION

Members of genus *Serratia* are soil-, air- and water-borne bacteria, some of them known to cause food spoilage (Lyhs et al. 1998; Berg, 2000; Sundaramoorthy et al., 2009). Serratias are also plant-associated organisms (Berg, 2000) and certain species were established as animal (Nieto et al. 1990) and human infectious agents (Okuda *et al*., 1981; Chmel**,** 1988; Carrero et al. 1995).

The habitats of *S. rubidaea* are not perfectly known. It has been isolated from raspberry, coconuts and from vegetable salads, but it has not been reported from water or animals (Ewing et al., 1973; Grimont and Grimont, 1991). However, *S. rubidaea* was isolated from clinical specimens, mainly from the respiratory tract or from ulcers or wounds (Johnson and Ellner, 1974; Ursua *et al*., 1996). Because there is no clinical information in relation to these isolates, *S. rubidaea'*s role in human disease has been dubious (Saito et al., 1989).

S. rubidaea is not known as a phytopathogen. This paper represents the first report of *S. rubidaea* causing bacteriosis of tulip bulbs.

MATERIALS AND METHODS

The strains included in this study originated from imported in Bulgaria diseased Holland tulip. The bulbs were with wrinkled, dried, cinnamon-colored tissues. Bacteria were isolated on King's medium B as described by Klement et al. (1990).

The pathogenic properties of the isolates were confirmed by infiltration of tobacco cv. Samsun NN (Klement et al., 1990) and by an artificial inoculation of onion bulb scales as described by Stoyanova et al. (2005). The presence of hypersensitive reaction (HR) was observed after 18 hours and the symptoms of the disease (change of the color, consistence, and turgor of tissues) were examined periodically between 1^{st}-5^{th} day.

Screening for major differentiating properties was performed by determining oxidase activity, Gram-reaction, anaerobic growth, motility, and cell morphology. Species identification of the isolates was carried out by the miniaturized identification system BIOLOG™ (BIOLOG™, USA) on the base of utilization of 95 carbon sources in GN2 MicroPlate™ test panels. The data analysis was accomplished with the software program MicroLog 4.20.05 (BIOLOG™, USA).

RESULTS

A total of 15 bacterial strains (isolates and reisolates) were investigated. The colonies had intense ruby color and thick, creamy texture. The bacteria were Gram-negative, oxidase-negative, facultative anaerobic motile rods which is characteristic for *Enterobacteriaceae* family.

All the strains gave hypersensitive reaction on tobacco leaves on the 18th hour and all caused disease in onion tissues on the 4th day after inoculation. The symptoms on onion scales were expressed as watery tissues with cinnamon color and sweet odor (Fig. 1).

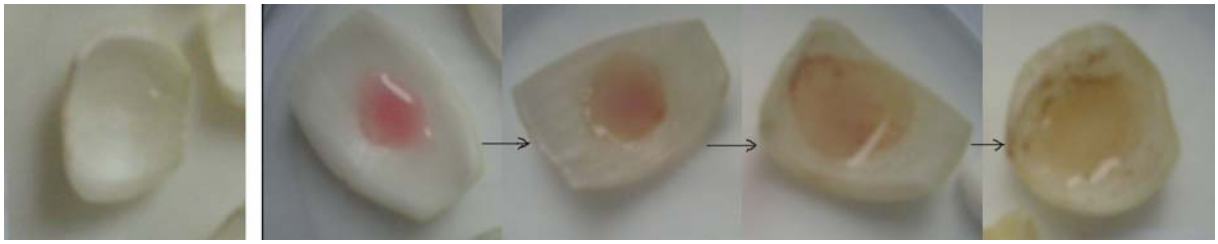

Fig. 1. A control scale and changes of onion tissues on the 2nd, 3rd, 4th, and 5th day after the artificial inoculation

All strains were identified with BIOLOG™ system as *Serratia rubidaea* (Stapp 1940) Ewing et al. 1973 (Tabl. 1). Fifty-six of the included substrates were utilized (dextrin, Tween 40, Tween 80, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, adonitol, L-arabinose, D-arabitol, i-erythritol, D-fructose, L-fucose, D-galactose, gentiobiose, m-inositol, α –D-lactose, maltose, D-mannitol, D-mannose, D-melibiose, b-methyl-D-glucoside, raffinose, sucrose, trehalose, turanose, pyruvic acid methyl ester, cis-aconitic acid, citric acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucuronic acid, p-hydroxy phenylacetic acid, α -keto glutaric acid, lactic acid, D-saccharic acid, succinic acid, glucuronamid, D-alanine, L-alanine, L-alanyl-glycine, L-asparagine, L-aspartic acid, glutamic acid, L-histidine, hydroxy-L-proline, L-phenylalanine, L-serine, γ-amino butyric acid, inosine, uridine, thymidine, putrescine, glycerol, glycerol phosphate, D-glucose-1-phosphate, D-glucose-6-phosphate) and fourteen of them were not utilized (α-cyclodextrin, β-hydroxybutiric acid, γ-hydroxybutiric acid, p-hydroxy phenylacetic acid, itaconic acid, α-keto butyric acid, α-keto valeric acid, propionic acid, sebacic acid, succinamic acid, L-leucine, L-proline, carnitine, 2-amino-ethanol, 2,3-butanediol). The strains differed in their reaction to 24 of the substrates (Table 2). The properties of the isolates were shared by their reisolates.

Table 1. Species identification with MicroLog™

Strain	ID (identification)	PROB (%) (probability)	SIM (similarity index)	DIST (distance)
6	*Serratia rubidaea*	100	0.552	7.05
6(1)	*Serratia rubidaea*	98	0.700	4.35
7	*Serratia rubidaea*	100	0.736	4.00
7(2)	*Serratia rubidaea*	99	0.655	5.24
7(3)	*Serratia rubidaea*	100	0.781	3.27
8	*Serratia rubidaea*	100	0.501	7.97
8(1)	*Serratia rubidaea*	100	0.779	3.31
8(3)	*Serratia rubidaea*	95	0.684	4.24
13	*Serratia rubidaea*	100	0.772	3.42
13(1)	*Serratia rubidaea*	100	0.657	5.24
14	*Serratia rubidaea*	100	0.647	5.42
14(1)	*Serratia rubidaea*	94	0.678	4.27
15	*Serratia rubidaea*	100	0.736	4.00
15(2)	*Serratia rubidaea*	100	0.797	3.00
15(3)	*Serratia rubidaea*	93	0.637	4.78

Table 2. Differences in BIOLOG™ substrates utilization

Substrate	Number of strains, utilizing the substrate*
Glycogen	(12)
D-cellobiose	15
D-glucose	13 (3)
Lactulose	10 (6)
D-psicose	(11)
L-rhamnose	(10)
D-sorbitol	1 (12)
Xylitol	(10)
Succinic acid mono-methyl ester	13 (1)
Acetic acid	3 (9)
Formic acid	7 (9)
D-glucosaminic acid	(9)
Malonic Acid	9 (1)
Quinic Acid	15
Bromosuccinic acid	12 (4)
Glycyl-L-aspartic acid	10 (3)
Glycyl-L-glutamic acid	10 (3)
L-alaninamide	9 (1)
L-ornithine	(5)
L-pyroglutamic acid	1
D-serine	9 (2)
L-threonine	11 (4)
Urocanic Acid	(12)
Phenylethylamine	(10)

* positive reaction (weak or delayed positive reaction)

DISCUSSION

Members of genus *Serratia* are soil-, air- and water-borne bacteria (Lyhs et al. 1998; Berg, 2000; Sundaramoorthy et al., 2009), plant-associated organisms (Berg, 2000), animal (Nieto et al., 1990) and human infectious agents (Okuda et al., 1981; Chmel**,** 1988; Carrero et al. 1995). However, *S. rubidaea'*s habitats are not perfectly known. The bacteria have been isolated first isolated from raspberry (Ewing et al., 1973), than from coconuts and vegetable salads, but not from water or animals. It was also found in a silastic foam dressing (Parment et al., 1984). The role of *S. rubidaea* as a human pathogen has not been proved (Grimont and Grimont, 1991) and as a plant pathogen it has not been reported.

From *Serratia* members only *Serratia marcescens,* known as environmental inhabitant, plant, animal, and human pathogen (Okuda *et al*., 1981; Escobar et al. 2001; Sundaramoorthy et al., 2009), was recently reported as a causal agent of bacteriosis of another bulb plant – onion (Escobar et al. 2001). Other species like *Serratia plymuthica* have been established both as animal pathogens and plant-associated organisms (Nieto et al., 1990; Carrero et al. 1995; Berg, 2000). This report, although being the first of *S. rubidaea* phytopathogenic strains, is not the first case when natural inhabitants, or human pathogens were also found as plant pathogens and the vice versa. The most typical examples are *Burkholderia cepacia* and *Burkholderia gladioli* first established as plant pathogenic bacteria and later as serious human infectious agents (Coenye & Vandamme, 2003). *Enterobacter cloacae* and *S. marcescens* represent another case when known environmental inhabitants and human pathogens were later established also as plant pathogenic bacteria (Bishop & Davis 1990; Falkiner, 1992; Escobar et al. 2001). Other bacterial species like *Pseudomonas aeruginosa, Pseudomonas fluorescens,* and *Pantoea agglomerans* are known to comprise saprophytic, human pathogenic and occasional plant pathogenic strains simultaneously (Starr & Chaterjee, 1972; Gibb et al., 1995; Schaad et al., 2001; Qarah et al., 2005). On the basis of the knowledge of these bacterial species, the results of our investigation on the background of the established plant-associated *S. rubidaea* strains and the probable human-associated *S. rubidaea* strains seem not exceptionable. The existing data (Govan et al., 2000; Escobar et al., 2001; Coenye & Vandamme, 2003; Neto et al., 2003) for the potential high adaptability or uniformity of natural, plant and human pathogenic strains in the frames of a single species suppose that factors for

pathogenicity may be much more complex than it was accepted before or that the increasing habitats for such bacteria may be a natural answer to the constantly growing anthropogenically affected regions.

The establishment of *S. rubidaea* in bulbs adds to the recent problem of constantly growing number of opportunistic pathogens and their habitats, and partially to the problem of their transfer.

CONCLUSIONS

Serratia strains with characteristics of *S. rubidaea* were found as phytopathogens of tulips. This paper represents the first report of *S. rubidaea* causing bacteriosis of tulip bulbs.

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