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SHORT COMMUNICATION

First Report of *Serratia plymuthica* Causing Onion Bulb Rot in Poland

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Abstract

Specific bacterial disease symptoms were observed on onion bulbs in almost all regions in Poland. For the purpose of identification of agents causing disease, bacteria were isolated from the symptomatic plants. Their pathogenicity was confirmed by using pathogenicity test on onion scales. These bacteria were identified biochemically and molecularly as *Serratia plymuthica*.

Key words: *Serratia plymuthica*, bacterial disease, onion

Onion (*Allium cepa* L.) is one of the major vegetable crops grown in Poland. Since the onion harvesting period often coincides with rainy weather and during cultivation it may hail, complex bacterial and fungal diseases often occur. In the last years especially bacterial diseases of onion cause very serious problems in Poland. These diseases may cause significant economic losses because they are difficult to control. Bacterial soft of onion bulbs is the most frequent and it can appear during cultivation, storage or transportation. It is common knowledge that in Poland, bacterial diseases of onion are caused by *Burkholderia gladioli*, *Burkholderia cepacia*, *Pectobacterium carotovorum* subsp. *carotovorum* (Sobiczewski and Schollenberger, 2002). Foreign reports conclude that bacteriosis of onion can also be caused by *Pseudomonas marginalis* (Kim *et al*., 2002; El-Hendawy, 2004), *Pseudomonas syringae*, *Pseudomonas viridiflava* (Gitaitis *et al*., 1998), *Pantoea ananatis* (Gitaitis *et al*., 2002), *Enterobacter cloacae* (Schroeder *et al*., 2009; Schwartz and Mohan, 2008), *Burkholderia ambifaria* and *Burkholderia pyrrocinia* (Jacobs *et al*., 2008). Also bacterium *Serratia* spp. was noted as an onion pathogen in Brasil (Beriam, 2007). The liberalization of policies concerning border protection and intense trade favor transmission of pathogens from foreign countries.

During the summer and autumn of 2003, 2006 and 2007, disease symptoms of unknown origin were observed on onion (*Allium cepa* L.) bulbs in the field and storage houses in different places in Poland. These symptoms were typical for bacterial disease – water soaked and pale brown lesions appeared on the internal scales, they enlarged and extended to external scales with an associated sour smell. Bacteria from the infected bulb tissues were isolated and purified on nutrient agar. About forty isolates were obtained and these isolates were examined for the ability to macerate onion tissue. The pathogenicity test was conducted on healthy onion bulbs *cv.* Grabowska. The bulbs were peeled, washed with running water and sterilized in 70% ethanol for 30 sec and in 0.5% NaOCl for 5 min. The bulbs were washed in sterile water and cut lengthwise into two parts. Three onion pieces were placed, cut side down, into a Petri dish (180 mm in diameter). The outer scale of each piece was wounded with the microbiological needle and inoculated by 20-µl aliquot of bacterial suspension of density $1.0–2.5 \times 10^8$ cfu/ml. For each isolate three Petri dishes were included. Control treatment remained uninoculated. The Petri dishes were incubated 4 days at 28°C. Nine of forty isolates expressed rot symptoms on the scales. These isolates were studied by using biochemical and molecular methods.

Biochemical identification of the isolates were performed by using the API 20E system (bioMerieux) which gave a bacterial code of 1207763 (Table I)

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Table I
Biochemical profile of tested isolates conducted using identification system API 20E

ONPG	ADH	LDC	ODC	CIT	H_2S	URE	TDA	IND	VP	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA	OX
+	–	–	–	+	–	–	–	–	+	+	+	+	+	+	–	+	+	+	+	–

test	**reactions / enzymes**
ONPG	β-galactosidase
ADH	arginine dihydrolase
LDC	lysine decarboxylase
ODC	ornithine decarboxylase
CIT	citrate utilization
H_2S	H_2S production
URE	urease
TDA	tryptophane deaminase
IND	indole production
VP	acetoin production
GEL	gelatinase
GLU	fermentation / oxidation (glucose)
MAN	fermentation / oxidation (mannitol)
INO	fermentation / oxidation (inositol)
SOR	fermentation / oxidation (sorbitol)
RHA	fermentation / oxidation (rhamnose)
SAC	fermentation / oxidation (saccharose)
MEL	fermentation / oxidation (melibiose)
AMY	fermentation / oxidation (amygdalin)
ARA	fermentation / oxidation (arabinose)
OX	cytochrome oxidase

(% identity = 59.6; T = 1.0). The numerical profile showed a correct identification of all examined strains as *Serratia plymuthica*. Additionally, some biochemical tests were conducted. The microorganisms were Gram-negative, catalase positive, oxidase negative and grew in anaerobic condition.

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AJ233433.1          CGTGTGTGAAGAAGGCCTTAGGGTTGTAAAGCACTTTCAGCGAGGAGGAAGGG--CAGTG
                    ||||||||||||||||||||||||||||||||||||||||||||||||||||  |||||
HM596429.1 361      CGTGTGTGAAGAAGGCCTTAGGGTTGTAAAGCACTTTCAGCGAGGAGGAAGGGTTCAGTG    420
                    ||||||||||||||||||||||||||||||||||||||||||||||||||||  |||
AY394724.1          CGTGTGTGAAGAAGGCCTTAGGGTTGTAAAGCACTTTCAGCGAGGAGGAAGGGTAGTGTG

AJ233433.1          TTAATAGCACAT-TGCATTGACGTTACTCGCAGAAGAAGCACCGGCTAACTCCGTGCCAG
                    ||||||||||  | | | ||||||||||||||||||||||||||||||||||||||||
HM596429.1 421      TTAATAGCAC-TGTRCATTGACGTTACTCGCAGAAGAAGCACCGGCTAACTCCGTGCCAG    479
                    ||||||||||  | ||||||||||||||||||||||||||||||||||||||||||||
AY394724.1          TTAATAGCACAT-TRCATTGACGTTACTCGCAGAAGAAGCACCGGCTAACTCCGTGCCAG

AJ233433.1          AGAA-TT-CGCTAGAGATAGCTTAGTGCCTTCGGGAACTCTGAGACAGGTGCTGCATGGC
                    ||||  ||  | |  ||||||  | ||  ||||||||||||||||||||||||||||||||
HM596429.1 960      AGAACTTTC-C-AGAGATGGATTGGTGCCTTCGGGAACTCTGAGACAGGTGCTGCATGGC    1017
                    ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AY394724.1          AGAACTTTC-C-AGAGATGGATTGGTGCCTTCGGGAACTCTGAGACAGGTGCTGCATGGC
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Fig. 1. Homology sequence alignment of the 16S rRNA region of type strain *S. plymuthica* DSM 4540 acc. no. AJ233433.1, tested isolate 466 acc. no HM596429.1, strain of the nearest BLAST match *S. plymuthica* RVH1 acc. no AY394724.1. Sequences were retrieved from the GenBank (National Center for Biotechnology Information) database under the accession numbers indicated.

Table II
BLAST search results for 16S rRNA gene sequence of representative isolate 466

S. plymuthica isolate	Accession no	Country	Identity	Match
DSM 4540 – type strain	AJ233433.1	Germany	98.9%	1449/1465
466 – tested isolate	HM596429.1	Poland	100%	1460/1460
RVH1	AY394724.1	Belgium	99.7%	1456/1461

The identity of the isolates was confirmed by molecular techniques – 16S rRNA sequence analysis. DNA was isolated by "Genomic mini" kit according to producent's clues (A & A Biotechnology). The 16S rDNAs were amplified by using universal primers fD1 (5'AGAGTTTGATCMTGGCTC3') and rP2 (5'ACGGCTACCTTGTTACGACTT3') (Weisburg *et al.*, 1991). Additional sequensing primers were used: 800f (5'ATTAGATACCCTGGTAG3') and 800r (5'CTACCAGGGTATCTAAT3') (Fouad *et al.*, 2002). The amplified PCR products, lenght 1500 bp, were separated by 1.5% agarose gel electrophoresis, then extracted and purified from gel with the DNA Fragment Purification Kit (A & A Biotechnology). DNA sequences were compared to NCBI database (www.ncbi.nlm.nih.gov) using BLAST program (Basic Local Alignment Search Tool), demonstrated that 16S rRNA gene of the studied isolates of bacteria shared high identities (99.7%) with *Serratia plymuthica* RVH1, NCBI GenBank database accession no. AY394724.1 (Fig. 1, Table II). The 16S rRNA sequence of the one representative isolate (466) has been deposited in the GenBank database under the accession number HM596429.1.

The biochemical test data along with sequence analysis of a portion of the 16S rRNA gene confirmed all the isolates to be *Serratia plymuthica*. According to our knowledge, this is the first report of *S. plymuthica* causing a bulb rot of onion in Poland.

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