PATHOGENIC FUNGI ASSOCIATED WITH SWEET POTATO AND THE BIOCONTROL POTENTIAL OF A *Bacillus subtilis* STRAIN

BOIU-SICUIA Oana-Alina, BARBU Lavinia Diana Nicoleta, COTEȚ George, PARASCHIV Alina

Abstract. The sweet potato, *Ipomoea batatas* (L.) Lam., is a highly productive crop, successfully acclimatized in Romania. Worldwide, in countries where sweet potato is a traditional crop, a wide range of plant pathogens is mentioned. In Romania, a few phytopathogenic infections have been encountered for this culture. However, *Fusarium* incidence on sweet potato increased in the last years. In the present paper the symptomatology of sweet potato fusariosis is described. The aim of the study is to reveal the biocontrol potential of a Romanian native strain of *Bacillus subtilis* in reducing *Fusarium* spp. growth. Bacterial physiologic studies have shown that the strain uses several mechanisms to inhibit fungal growth. *In vitro* tests showed an efficiency of 31.3 to 51.4% for the growth inhibition of phytopathogenic fusaria.

Keywords: *Bacillus subtilis*, biocontrol, *Fusarium*, sweet potato.

Rezumat. Fungi patogeni asociați cartofului dulce și potențialul de biocontrol al unei tulpini de *Bacillus subtilis* Strain. Cartoful dulce, *Ipomoea batatas* (L.) Lam., este o cultură extrem de productivă, aclimatizată cu succes în România. La nivel mondial, în țările cu tradiție pentru cultivarea cartofului dulce, este menționată o gamă largă de agenți fitopatogeni. În România, spectrul de fitopatogeni la această cultură este mai restrâns. Incidența fuzariozei la cartoful dulce a crescut însă în ultimii ani. În prezenta lucrare este descrisă simptomotologia fuzariozei la cartoful dulce. Scopul studiului este de a evidenția potențialul de biocontrol al unei tulpini autohtone de *Bacillus subtilis* pentru reducerea creșterilor de *Fusarium* spp. Studiile de fiziologie microbiană au arătat că tulpina prezintă mai multe mecanisme de inhibare a creșterii fungilor. Testele *in vitro*, au arătat o eficacitaste de inhibare a creșterii fuzariilor fitopatogene de 31,3 până la 51,4%.

Cuvinte cheie: *Bacillus subtilis*, combatere biologică, *Fusarium*, cartof dulce.

INTRODUCTION

The sweet potato, *Ipomoea batatas* (L.) Lam., belongs to the Convolvulaceae family, being native to the tropical regions of South America. However, it is now cultivated over large areas in Africa, Asia, America, Oceania and some Mediterranean areas of Europe. Worldwide, the sweet potato is cultivated on more than 7.8 million ha, with an average yield of 11.8 t/ha, in 2019. According to FAO, in 2019, the larger growth areas are in Africa, on 4.4 million ha, followed by Asia, on 2.9 million ha, especially in China, India and Japan. In America, larger areas cultivated with sweet potato are in the South, but productivity is higher in the North, the average yield in the USA being 24.4 t/ha. Still, a higher productivity is encountered in some African countries, such as Senegal (38.6 t/ha), Ethiopia (33.5 t/ha), or Egypt (32.1 t/ha). Australia is also having good yields, being the second country in the world in terms of productivity per hectare, with an average yield of 36.4 t/ha. However, the main producer worldwide is China, which provides more than 50 million t / year, as it was shown in the last 20 years (www.fao.org).

According to FAO (2017), in Europe, sweet potato is cultivated mainly in Spain, Portugal, Italy and Greece (Table 1). The species was successfully acclimatized also in Romania, where it maintains its highly productive potential (DIACONU et al., 2018).

Table 1. Statistics regarding sweet potato crop in Europe (according to the Food and Agriculture Organization of the United Nations, 2017).

No.	European Country	Harvested area <ha>	Average yield <t/ha>
1	Spain	2145	29.05
2	Portugal	954	24.32
3	Italy	388	21.89
4	Greece	90	23.92

In recent years, the interest in consuming sweet potatoes is increasing. This is due to its nutritional content and beneficial traits, especially as baby food, and in different diets, especially for diabetics. Sweet potato contains high level of carbohydrates, antioxidants such as carotenoids (which converts into vitamin A once consumed) and vitamin C, and represent a source of dietary fiber and minerals (K, Fe, Ca, Na, Zn, Mg, P, Se) (SANOUSSI et al., 2016).

In the last few years, an increased interest in growing sweet potato was seen in Romania. This is mostly due to the high productivity, good selling price and reduced phytosanitary problems. In our country, *Fusarium* infections are some of the most damaging diseases of sweet potato. These pathogens can cause up to 50% damage to the sweet potato crop (CLARK, 1987). Several control methods are mentioned for these pathogens. Conventional growers can use azoxystrobin and fludioxonil as active chemical ingredients (https://content.ces.ncsu.edu). Although such pesticides are approved to be used in the UE, including Romania (http://ec.europa.eu), there is no data referring to their application for sweet potato. Other chemical active substances, such as thiabendazole, or other benzimidazoles, are also mentioned to be effective against fusariosis on sweet potato.

Fusarium disease control of sweet potato is also possible for organic growers. In the USA, a mixed treatment with hydrogen dioxide and peroxyacetic acid (Oxidate 2.0) can be used. Active substances of plant origin, such as *Reynoutria sacchalinensis* extracts (Regalia), or biocontrol agents, such as *Streptomyces lydicus* WYEC 108 (Actinovate AG) are also mentioned for biological control. Bacillus biocontrol agents such as *Bacillus safensis* T052-76, *B. velezensis* T149-19, and selected strains of *B. subtilis* species complex, are also mentioned to protect sweet potato against important pathogens (MARQUES et al., 2015; MATEUS et al., 2019).

Various symptoms of fusariosis, on several cultivars of sweet potato grown on sandy soils in Dolj County, Romania, are presented in this study. The aim of the study is to reveal the biocontrol potential of a *Bacillus subtilis* selected strain in reducing *Fusarium* spp. growth.

MATERIAL AND METHODS

Plant material. Six cultivars of sweet potato (Table 2, Fig. 1), *Ipomoea batatas* (L.) Lam., cultivated in the sandy soils of Research-Development Station for Field Crops on Sandy Soils – Dăbuleni (Dolj county, Romania) were analysed for phytopathogenic infections caused by *Fusarium* species. The study was carried out during the growing season of 2020.

Table 2. Analysed cultivars of sweet potato.

No.	Sweet potato	Tubers
1	KSP1 variety	Purple-Red skin, yellowish flesh
2	DK 19/1 biologic line	Pink skin, white flesh
3	DK 19/2 biologic line	Purple-Red skin, yellowish flesh
4	DCh 19/3 biologic line	Dark purple skin and flesh
5	DK 19/4 biologic line	Red skin, white flesh
6	DK 19/5 biologic line	Pinkish skin, peachy-orange flesh

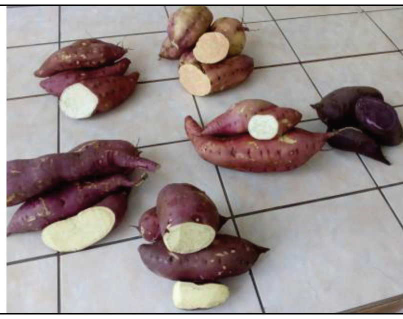

Figure 1. Tubers of sweet potato.

Plant pathogens analysis. Infected plant material was collected from the diseased plants. Samples were subjected to laboratory analysis, under the stereomicroscope and transmitted light microscope, in order to confirm the pathogenic infection. Fungi were isolated in pure cultures and microbiological characterized.

Biocontrol bacteria. *Bacillus subtilis* Dj3 strain was used as biocontrol agent. The strain was grown in laboratory-scale production system in order to obtain a high yield of bacterial biomass for agro-inoculant production. Biomass samples were collected and biological activity was verified *in vitro*, compared to its previous traits (BOIU-SICUIA et al., 2017).

Fungal antagonism. The double culture technique was used to evaluate the antagonistic potential of *B. subtilis* Dj3 strain against newly isolated pathogenic fungi. Tests were performed on the PDA (Potato-Dextrose-Agar) medium. Bacterial biomass was streaked on the medium at 2 cm distance from the fungal inoculum. Tested fungi were calibrated as mycelial plugs of 5 mm diameter. Fungal control plates were also prepared, for each of the tested fungi. Tests were performed in duplicate. Plates were incubated at 28°C and periodically analysed up to 14 days.

RESULTS AND DISCUSSION

Sweet potato pathogenic infections

Inspections made in the experimental field of sweet potato grown on sandy soils at SCDCPN Dăbuleni (Dolj county, Romania), during the growing season of 2020 revealed a reduced phytopatogenic attack (Fig. 2).

Among tested cultivars of sweet potato, the most resistant to plant diseases was the D-Ch 19/3 biological line, in which the pathogenic attack degree was 0.68 % ± 0.08. The most sensitive cultivar was the DK 19/5 biological line, where the attack degree was 2.64 % ± 0.3. Compared to the growing season of 2016, the incidence of fusariosis increased in the SCDCPN Dăbuleni fields of sweet potato (BOIU-SICUIA et al., 2017).

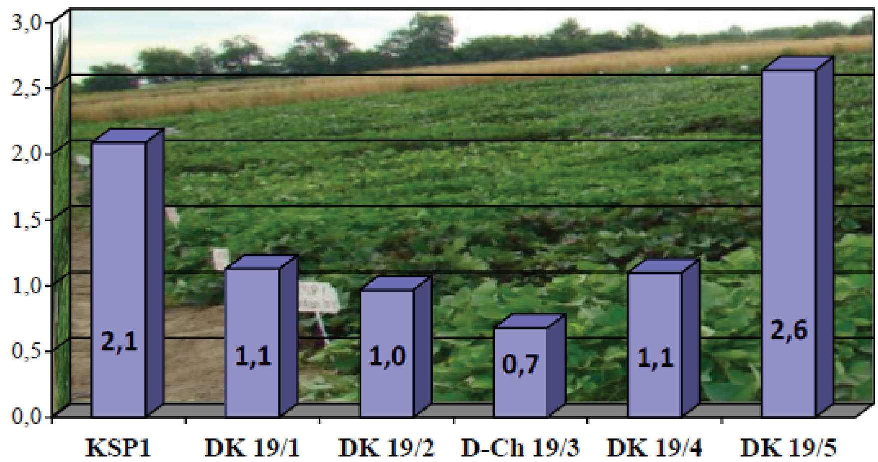

Figure 2. Phytopatogenic attack degree (%) in different cultivars of sweet potato.

According to the symptomatology only some infections were caused by fusariosis. The infected plants revealed yellowing of the older leaves, and stunting of the vine growth. Discoloration of the vascular tissue was observed. When the cortex was removed or the infected stems were cut, some of the xylem vessels were brown in the cross section. In humid chamber, under laboratory conditions, sporulated mycelia were developed on the diseased plant parts (Fig. 3a). Harvested infected tubers also developed fungal growth (Fig. 3b). Microscopic analysis of the fungal growth revealed septate hyphae and abundant conidia of various shape. Microconidia are ovoid, while macroconidia are sickle-shaped and septate (Fig. 3c). Such microscopic characteristics are correlated to *Fusarium* species.

Figure 3. *Fusarium* sp. isolated from sweet potato plants
a. infected stem seen under the stereomicroscope, b. infected potato tuber, c. conidia from infected plant tissue.

Studies regarding *Fusarium* sp. infection in sweet potato mention the Fusarium root rot as caused by *F.oxysporum*, and Fusarium wilt as caused by *F.oxysporum* f. sp. *batatas*. Although, they cannot be distinguished by morphology, there are differences in pathogenicity. Surface rot of storage roots is mostly associated to *F. solani*. f. sp. *batatas* (CLARK & MOYER, 1988). More recent studies on Fusarium root rot, mention *F. solani* (CHAI et al., 2007; SCRUGGS & QUESADA-OCAMPO, 2016; YANG et al., 2018) and *F. proliferatum* (DA SILVA & CLARK, 2013; SCRUGGS & QUESADA-OCAMPO, 2016) as causal organisms. Several other species were revealed to infect sweet potato, such as *F. acuminatum* (FARR & ROSSMAN, 2006; SCRUGGS & QUESADA-OCAMPO, 2016), *F. circinatum* (YANG et al., 2019), *F. denticulatum* (GONZÁLEZ et al., 2003), even *F. graminearum* (SCRUGGS & QUESADA-OCAMPO, 2016) and other stains, like *F. meridionale* and *F. lateritium* of Graminaerum clade. Still, the attack of *F. oxysporum* remains the most frequent cause (PAUL et al., 2020; SCATTOLINI et al., 2020) and *F. solani* (YANG et al., 2018).

Bacillus subtilis **Dj3 strain**

The biomass production of the *Bacillus subtilis* Dj3 strain was obtained from 3 days' old submersed cultures, by centrifugation. Before the formulation as alginate beads, the biological activity was checked. Bacteria revealed the same plant beneficial and biocontrol traits (Table 3, Fig. 4).

Table 3. Biological activity of *Bacillus subtilis* Dj3.

Figure No	Test	Biologic activity results	Relevance	
4.a	Swimming motility	+ (in less than 24h)	Swimming motility is correlated to cell migrations and bacterial spreading capacity	
4.b	Swarming motility	+ (in less than 24h)	Swarming motility is correlated to the colonization capacity and biofilm formation	
4.c	Chitinase production	+ (after 3 days)	Chitinase production is correlated to fungal cell wall lysis	Enzymes contributing to

4.d	Cellulase production	+ 0.5 cm (after 5 days)	Chitinase production is correlated to: - oomycete cell wall lysis - elicitation of plant defense mechanism	fungal antagonism
4.e	Caseinase production	+ 0.5 cm (after 3 days)	Protease production is correlated to the fungal cell membrane degradation	
4.f	Gelatinase production	+ 1.6 cm (after 3 days)		
4.g	Acetoin production	+ (after 3 days)	Acetoin is a volatile organic compound involved in plant protection & growth stimulation	
4.h	Amylase production	+ 0.6 cm (after 3 days)	Amylase production is correlated to gibberellin synthesis and therefore with in plant growth stimulation	Enzymes contributing to plant growth promotion
4.i	Arginin-decarboxilase production	+ (in less than 24h)	Arginin-decarboxilase is correlated to polyamines synthesis and protection against environmental stress factors (in both bacteria and inoculated plants)	
4.j	IAA production	9.48 µg/ml	Phytohormone involved in plant growth stimulation	
4.k	Phosphorus solubilisation	+ (after 7 days)	Phosphate solubilization is involved in plant growth promotion. Moreover, improved phosphorus uptake increases plant tolerance against some environmental stress factors.	

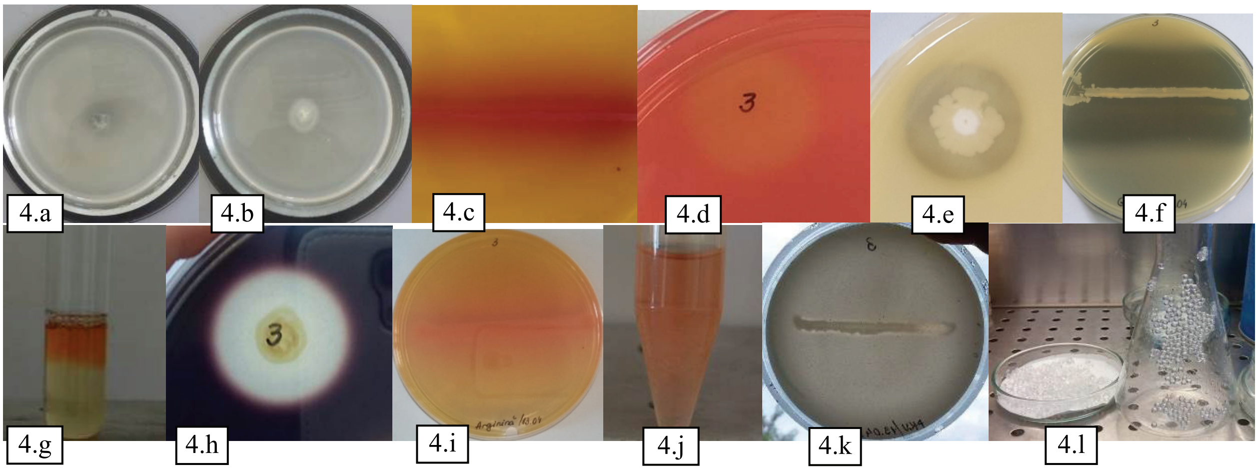

Figure 4. Biological activity of *Bacillus subtilis* Dj3 (a ÷ k) and formulation as alginate beads.

Antifungal activity of *Bacillus subtilis* Dj3

The antifungal activity of *Bacillus subtilis* Dj3 strain was evaluated against seven strains of *Fusarium* sp. isolated from sweet potato fields. The antifungal activity was biometrically evaluated, and clear inhibition zone were measured after 5 days of incubation at 28°C (Fig. 5, Table 4).

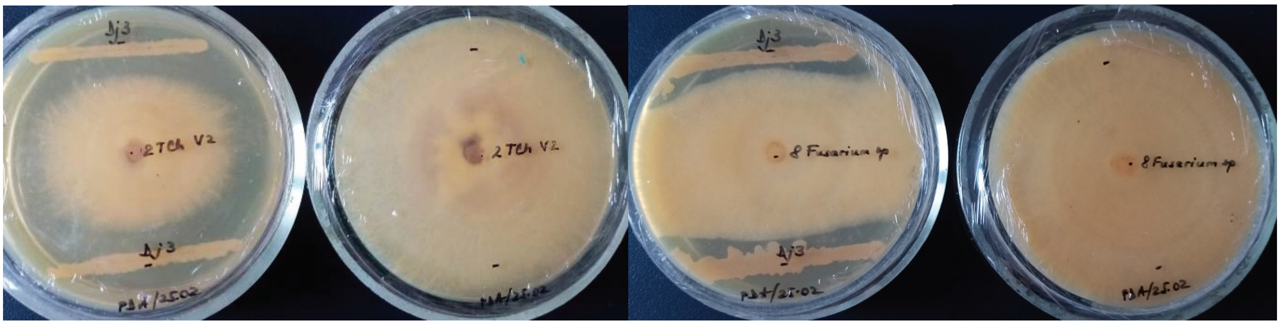

Figure 5. Antifungal activity of *Bacillus subtilis* Dj3 against sweet potato pathogenic fusaria.

Table 4. Antifungal activity of *Bacillus subtilis* Dj3 against sweet potato pathogenic fusaria. (after 5 days of co-cultivation at 28°C).

***Fusarium* sp. pathogens of sweet potato**	***Fusarium* sp. isolation source**	**Clear inhibition zone (cm)**	**Antagonistic efficacy**
Isolate 1 TBiol/V3.1	Sweet potato DK 19/2 line organically grown	0.1	38.2 %
Isolate 2 TChV2	Sweet potato DK 19/1 line conventionally grown	0.5	51.4 %
Isolate 3 TCh +BioV2	Sweet potato DK 19/1 line conservative grown	0.1	37.1 %
Isolate 6 TChV6	Sweet potato DK 19/5 line conventionally grown	–	31.3 %
Isolate 8	Sweet potato ROK1 variety conventionally grown	0.1	43.5 %
Isolate 9 TCh+BioV2	Sweet potato DK 19/1 line conservative grown	0.1	45.7 %
Isolate P10	Sweet potato Juhwangmi variety conventionally grown	0.1	32.4 %

Best results were obtained against *Fusarium* sp. isolate 2. The bacteria maintained a clear inhibition zone of 5 mm towards the fungal colony. *In vitro,* the antagonism efficacy was evaluated as 51.4.1%. A moderate antifungal effect was also seen against *Fusarium* sp. isolates no. 8 and 9, where the calculated inhibition efficacy ranged from 43.5 to 45.7%, followed by isolates no. 1 and 3, where the inhibition efficacy was among 38.2 and 37.1%,

A reduced inhibition potential was seen against *Fusarium* sp. isolates 6 and P10 collected from conventionally grown DK 19/5 biological line and Juhwangmi variety of sweet potato. According to the field inspection, DK 19/5 cultivar was most sensitive to phytopathogenic infections.

CONCLUSIONS

Fusarium incidence on sweet potato increased in the last years. Therefore, new strategies for plant protection should be considered in order to prevent further infections. For the organic growth of sweet potato, biocontrol microorganisms can be considered. Selected *Bacillus subtilis* biocontrol strains tested *in vitro* can be considered as potential preventive measures in order to reduce *Fusarium* spp. infection rates.

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Boiu-Sicuia Oana-Alina, Barbu Lavinia-Diana-Nicoleta
Research and Development Institute for Plant Protection, 8 Ion Ionescu de la Brad Blvd., Bucharest, Romania.
University of Agronomical Sciences and Veterinary Medicine Bucharest, Faculty of Biotechnologies, 59 Mărăști Blvd, Bucharest, Romania.
E-mails: sicuia_oana@yahoo.com; laviniabarbu7@gmail.com

Coteț George, Paraschiv Alina
Research-Development Station for Field Crops on Sandy Soils - Dăbuleni,
217 Petre Baniță str., Călărași Village, PC 207220, Dolj County, Romania.
E-mails: georgecotet1968@gmail.com; alina22paraschiv@yahoo.com

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