RESEARCH APPROACHES REGARDING BIOLOGICAL CONTROL OF *Fusarium* sp. STEM ROT OF SWEET POTATO PRODUCED ON SANDY SOILS

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Abstract. The sweet potato *Ipomoea batatas* (L.) Lam. is a world wide appreciated vegetable due to its nutritional value. It is a member of the Convolvulaceae family, originated from the tropical areas of America. In Romania, the sweet potato is less known for both growers and consumers. However, at the Research-Development Centre for Field Crops on Sandy Soils (RDCFCSS) – Dăbuleni, this vegetable has been successfully introduced in the crop rotation for almost five years; the crop technology was adapted and improved for the sandy soils conditions from the southern Oltenia. For this crop, the spectrum of plant diseases is, however, reduced in our country, with no management problems or economic important losses before harvest. However, in 2016, one case of Fusarium stem rot was noticed for the first time in the field. The infected plant was visibly affected and its production potential was reduced to half. Although the infection was limited to a single plant and did not spread during the whole vegetation season, the severity of the attack triggered our concern. The aims of this study was the identification of the pathogenic infection as there are similarities between the symptoms of disease and detect some biological means to suppress the evolution of such an infection. Thirty bacterial strains were isolated for this purpose, from the rhizosphere of sweet potato grown in the same field, and two *Bacillus* sp. strains expressed high antagonistic activity, in vitro, against *Fusarium* stem rot of sweet potato.

Keywords: *Ipomoea batatas*, *Fusarium* stem rot, biocontrol bacteria.

Rezumat. Studiu privind combaterea biologică a putregaiului tulpinilor cauzat de *Fusarium* sp. la cartoful dulce cultivat pe soluri nisipoase. Cartoful dulce *Ipomoea batatas* (L.) Lam. este o legumă apreciată în întreaga lume datorită proprietăţilor nutritive. Specia face parte din familia Convolvulaceae, originară din zonele tropicale ale Americii. În România, cartoful dulce este mai puţin cunoscut atât pentru cultivatori, cât şi pentru consumatori. Cu toate acestea, la Centrul de Cercetare-Dezvoltare pentru Cultura Plantelor pe Nisipuri - Dăbuleni această plantă este cultivată cu succes de aproximativ cinci ani, tehnologia de cultură fiind adaptată şi îmbunătăţită pentru condiţiile solurilor nisipoase din sudul Olteniei. În prezent, spectrul de boli la această cultură este redus în ţara noastră, iar menţinerea sub control a bolilor nu creează dificultăţi sau pierderi economice înainte de recoltare. Cu toate acestea, în 2016, a fost detectată o plantă cu putregai la nivelul tulpinii. Planta infectată era vizibil afectată, iar potenţialul de producţie i-a fost redus la jumătate. Chiar dacă infecţia era izolată, şi nu s-a extins la plantele adiacente pe tot parcursul sezonului de vegetaţie, severitatea atacului este îngrijorătoare. Scopul acestui studiu este acela de a identifica patogenul care a cauzat simptomele de boală şi de a găsi o soluţie pentru combaterea biologică a eventualelor infecţii. Pentru aceasta au fost izolate treizeci de rizobacterii din aceeaşi cultură obţinută pe terenuri nisipoase. Dintre acestea, două tulpini de *Bacillus* sp. au prezentat activitate antagonistă, in vitro, faţă de specia de *Fusarium* care a cauzat putrezirea tulpinilor de cartof dulce.

Cuvinte cheie: *Ipomoea batatas*, fuzarioza tulpinilor de cartof dulce, bacterii de biocontrol.

INTRODUCTION

The sweet potato (*Ipomoea batatas*) is a world wide appreciated vegetable due to its nutritional value. It is rich in complex carbohydrates, minerals (P, K, Ca and Na), vitamins (mostly vitamin C, B5 and B6) and carotenoids, especially in orange and purple fleshed varieties (PARLE, 2015). It is also considered a medicinal plant with anti-cancer and anti-inflammatory activity (SANDHYA et al., 2011). Due to its lower glycaemic index sweet potatoes are also suitable in diabetes diet, as it slowly releases glucose into the bloodstream and raise the blood levels of adiponectin, which helps the body to metabolize insulin (DUTTA, 2015).

This plant is mainly grown for tubers production, although in some Asian counties, in the human diet, there are used both tubers and sprouts from the sweet potato plants. However, it can be also found as ornamental plant, improving the landscape architecture of outdoors public areas, where it is associated with other ornamentals, in big pots or containers.

In our country, the sweet potato was experimentally introduced by Maier I. in 1954, and formally studied by CIOFU et al. (2004) and MUȘAT (2013) at the Faculty of Horticulture, University of Agronomic Sciences and Veterinary Medicine Bucharest. Two Romanian varieties have been obtained through conservative selection process, Victoria IANB and Crux (CIOFU et al., 2004). These two varieties were available on the market until 2008 and 2009, respectively (Official Monitor of Romania – Part I, 2007). From 2015, the Korean company Love of Soil Machinery Co. LTD managed to register other two varieties in Romania, KSC1 (South Korean Chestnut) and KSP1 (South Korean Pumpkin), which are now listed in the Official Catalogues of ISTIS (State Institute for Variety Testing and Registration). The registration of these varieties was sustained by the "Romanian-Korean Collaborative partnership for the sweet potato", established between Kyungpook National University (KNU) in South Korea and the Academy of Agricultural and Forestry Sciences "Gheorghe Ionescu Șișești" Bucharest (DIACONU et al., 2016). Based on this collaboration protocol, since 2012, research on sweet potato culture has been carried out in southern Oltenia, at Research-Development Centre for Field Crops on Sandy Soils (RDCFCSS) Dăbuleni. Currently, the sweet potato is considered a valuable crop for the pedoclimatic conditions of that area. To our knowledge, the sweet potato is now

cultivated on small areas in counties like Argeş, Brăila, Caraş-Severin, Cluj, Dolj, Gorj, Hunedoara, Ilfov, Olt, Prahova, Satu Mare and Vaslui (DIACONU et al., 2016).

Ipomoea batatas is a thermophilic plant, drought tolerant, which grows well on medium fertile soils, well drained with loose structure. In our country, the sandy soils of southern Oltenia region offer favourable pedoclimatic conditions for sweet potato crop. Therefore, during the last five years, the RDCFCSS Dăbuleni managed to acclimatize some Korean varieties of sweet potato, and also implemented and improved the culture technology of this crop in our country. During this time the phytosanitary aspects in the field did not raise crop management problems or economic looses before harvest, although large quantitative losses occurs during storage. However, in 2016, one case of *Fusarium* stem rot was noticed for the first time in the field. Although the infection was limited to a single plant, and did not spread during the whole vegetation season, the severity of the attack triggered our concern. Therefore, one of the aims of this study was to identify the pathogen inducing the stem rot as there are similar symptoms produced by different plant pathogens. Another goal was to suppress the evolution of such an infection using some native biological means.

In our study we isolated thirty bacterial strains from the sweet potato rhizosphere of the plants grown in the same field, with sandy soil conditions. Two *Bacillus* sp. strains were selected based on their high antagonistic activity, *in vitro*, against *Fusarium* stem rot of sweet potato.

MATERIAL AND METHODS

Phytopathogenic fungi. Several Korean varieties of *Ipomoea batatas* were experimentally grown in 2016, at the RDCFCSS Dăbuleni, Dolj County, Romania (Fig. 1). These five varieties, KSC1, KSP1, Hayanmi, Juhwangmi and Yulmi, were each grown on 10 square meters, using 30 plants/ variety.

Figure 1. Korean varieties of *Ipomoea batatas* experimentally grown in 2016, at RDCFCSS Dăbuleni (original).

In Hayanmi variety, one plant with symptoms of wilts and stem rot was detected (Fig. 2a, b). The leaves colour modified from green to reddish-purple and yellow (Fig. 2a). The main vein was dry starting from the base of the plants, the stem progressively turned black and it was easily broken by hand. Necroses of the vascular bundle were seen (Fig. 2b) and therefore, the plant material was taken in the laboratory for further analysis and pathogen identification.

Figure 2. Wilting and dry stem rot of sweet potato plant from Hayanmi variety grown on the sandy soil of southern Oltenia (2016): **a.** abnormal leaves colour, and **b**. dry stem rot of sweet potato (originals).

Beneficial bacteria. Soil samples from the sweet potato culture grown in the sandy soils of RDCFCSS Dăbuleni were analyzed in order to isolate indigenous bacterial strains with antagonistic activity against *Fusarium* stem rot of *Ipomoea batatas*. The performed isolation protocol was according to BOIU-SICUIA et al. (2016).

Purification and characterization of the isolates was carried out by growing the bacteria on different culture media. For colony morphology description, each strain was grown on LB agar using the streak plate method to obtain isolated colonies. To evaluate the oxygen requirement each strain was grown on nutrient broth in static incubation. Gram reaction was carried out using the 3% KOH test.

Antagonism evaluation. The antagonistic activity of the isolated bacteria was evaluated *in vitro* using the dual-culture assay. The test was performed on PDA media against *Fusarium* stem rot of *Ipomoea batatas*. The antifungal activity was calculated according to ISLAM et al. (2009).

Plant beneficial bacterial tests. The isolated bacterial strains were analyzed for their plant beneficial traits and biocontrol mechanisms. The phytohormone production was quantified on Luria Bertani (LB) medium and LB supplemented with 5mM tryptofan, as precursor of auxin (PATTEN & GLICK, 1996). Several enzymes production, like amylase, phosphatase, cellulase, chitinase, and proteases were analyzed (SICUIA et al., 2015), as they are correlated with plant growth promotion or plant protection. Bacterial swimming and swarming motility was also evaluated as it correlates with plant root colonization and pathogen competition for the niche (CONSTANTINESCU et al., 2010).

Microbial identification. The infected plant material was maintained in humid chamber for almost one month. The fungal growth developed on the sweet potato stem was analyzed under the binocular magnifier and then was purified on Potato–Dextrose-Agar (PDA) medium. The identification was made using two laboratory procedures. At first, there were used classical methods based on colony morphology and microscopic characteristics, and then the Biolog identification system for filamentous fungi was used according to the standard protocol. The antagonistic bacterial strains were identified based on their phenotypic and biochemical characteristics using the Biolog GEN III identification system.

RESULTS AND DISCUSSION

During the vegetation season of 2016, five Korean varieties of *Ipomoea batatas* were experimentally grown at RDCFCSS Dăbuleni. One case of *Fusarium* stem rot was noticed during the whole season of 2016, at a single plant, in Hayanmi variety. The incidence of disease in this variety was 3.33%, and 0.66% in the experimental plot. Although the infection was limited to a single plant and did not spread during the whole vegetation season, the severity of the attack triggered our concern. Therefore, the pathogen identification was carried out in laboratory conditions.

After one month of incubation at room temperature in the humid chamber, the pathogen developed white cottony mycelia on the sweet potato stem (Fig. 3a). The microscopic analysis showed a septate filamentous fungus (Fig. 3b) with typical *Fusarium* like macroconidia (Fig. 3c).

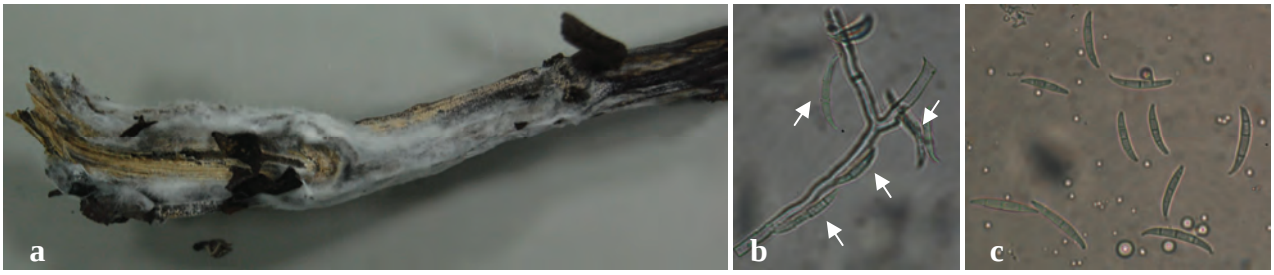

Figure 3. *Fusarium* stem rot of sweet potato: **a**. infected stem, **b**. septate mycelium with conidia (arrows), and **c.** *Fusarium macroconidia* with three-septa, and slight dorsi-ventral curvature (originals).

Pure cultures were obtained on PDA (Potato Dextrose Agar), MA (Malt extract Agar) and CYA (Czapek Yeast extract Agar) culture media. On PDA, the fungal colony was flat, cottony with purple pigmentation (Fig. 4a), and on MA the pigmentation was reddish (Fig. 4b) and the growth rate was much slower. Unlike these, on CYA medium, the pathogen grew abundantly and much faster, and developed woolly and buttery coloured colonies (Fig. 4c). On PDA medium, the pathogen produced both micro- and macroconidia, and also chlamydospores. However, on MA the sporulation was poor, but on CYA growth medium conidia production was highly stimulated, but only micro- and mesoconidia were produced.

Analyzed with the Biolog system for filamentous fungi, the pathogen was identified as belonging to *Fusarium* genus, the most similar species being *F. subglutinans*, *F. verticillioides* and *F. oxysporum* (Fig. 5, Table 1).

Both microbiologic and biochemical analysis revealed that the pathogen is affiliated to *Fusarium* genus. The Biolog FF identification is influenced by the metabolic reaction of the fungi grown in 95 carbon source kit, were substrate oxidation and cell growth are measured spectrophotometrically and compared with the database collection. Therefore, due to strain characteristics there can be found some differences in the time consumption of the culture substrate that can confuse the

identification or fungi similarity from one day to another (Table 1). However, the fungal species revealed as similar with our *Fusarium* sp. strain are basically the same: *F. subglutinans*, *F. verticillioides* and *F.oxysporum.* The macro and microscopic analysis leads to *F. subglutinans* but atypical to the characters of this species, the studied strain of *Fusarium* sp. can form chlamydospores, a character that does not exclude membership in *F. oxysporum* species. Despite uncertainty at species level, *Fusarium* spp. pathogens can cause particular problems in agricultural crops. Although in our country the sweet potato culture is not economically affected by fusariosis, the presence of *Fusarium* sp. pathogens suggests the importance of finding a cure for this disease before it becomes devastating.

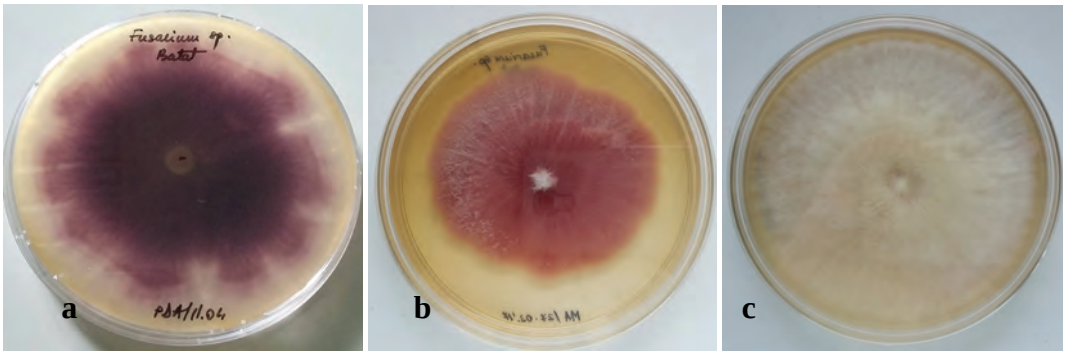

Figure 4. *Fusarium* colony morphology on: **a**. PDA, **b**. MA, and **c.** CYA growth media (originals).

Table 1. *Fusarium* biochemical similarities revealed by the Biolog identification system for filamentous fungi.

***Fusarium* species matches**	**SIM values at specific incubation times required for filamentous fungi identification**			
	24h	**48h**	72h	**168h**
F. subglutinans (Wollenweber & Reinking) P.E.Nelson BGB	-	-	0.066	**0.491**
F. verticilloides (Saccardo) Nirenberg BGE	-	0.104	**0.428**	0.288
F. oxysporum Schlechtendahl: Fries BGB	-	**0.372**	-	0.007
F. sacchari (Butler) W.Gams BGA, current name and common synonym ***F. subglutinans*** (Wollenw. & Reink) Nelson	**0.346**	0.097	0.038	-
F. pseudoanthophilum Nirenberg & O'Donnell	-	0.153	-	-
F. subglutinans (Wollenweber & Reinking) P.E.Nelson BGA	0.026	-	0.021	-
F. verticilloides (Saccardo) Nirenberg BGA	-	-	-	0.010
F. sambucinum var. *sambucinum* Fuckel	0.005	-	-	-
F. udum E.Butler	0.002	-	-	-
Sum of the SIM	0.379	**0.726**	0.553	**0.796**
Note: For species identification the similarity index value (SIM) must be ≥ 0.9 at 24h of incubation, ≥ 0.7 at 48h of incubation, and ≥ 0.65 at 72h of incubation. If SIMs are under the values required, but all species listed belong to the same genus and their combined SIMs gives a value above the one recommended then the MicroLog identification system will give the confirmation that the analyzed microbe is in the listed genus.				

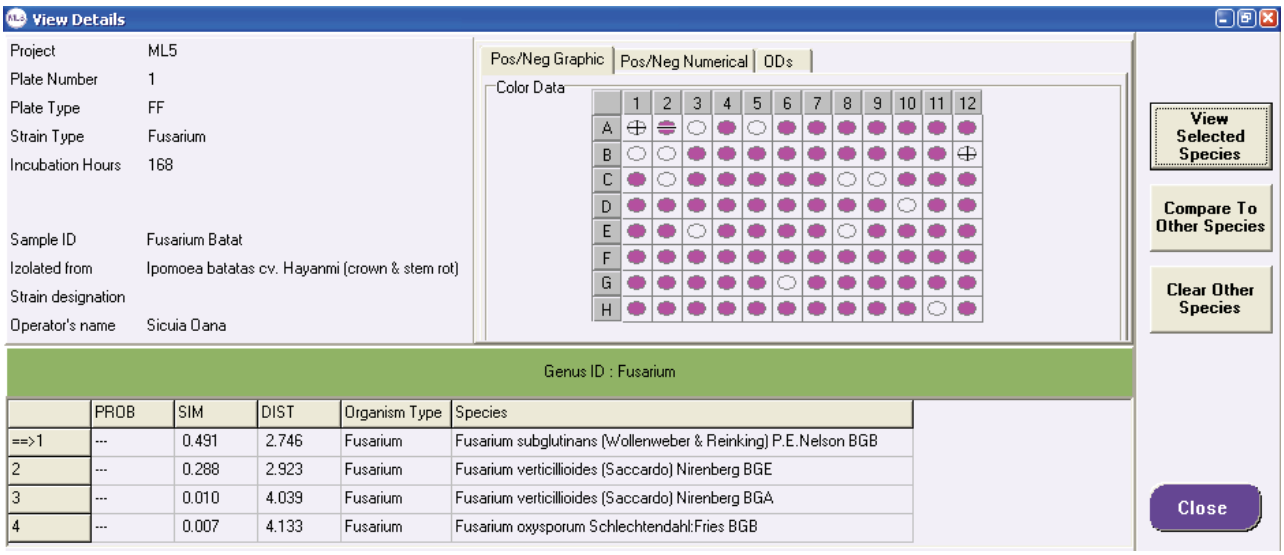

Figure 5. *Fusarium* phenotypic identification with the Biolog system for filamentous fungi.

Comparing the symptoms of disease, the macroscopic and microscopic characteristic of the pathogen pure cultures and the nutritional preferences revealed by the Biolog system, the pathogen was identified as *Fusarium* sp. Taking into account that sweet potato is susceptible to *Fusarium* stem rot (OKADA et al., 2017), the management of this disease should be taken into consideration. In our study, the infected plant expressed an index disease of 3, according to OGAWA et al. (1979) disease scale. Therefore, preventing the sweet potato plants from this pathogen is an important issue that must be solved. In our study, we searched for biological control agents. In this regard, we isolated several bacteria from the rhizosphere of healthy plans. Thirty bacterial strains were purified, 17 of them being Gram positive (Table 2).

When analyzed for their plant growth promoting ability, 15 strains revealed phosphatase activity on Pikovskaya agar medium, after 7 days of incubation at 27°C. Amylase activity, correlated with gibberellins production, was revealed by 13 strains, when bacteria were grown for three days on nutrient agar medium supplemented with 4% soluble starch (Table 2).

Table 2. Characteristics of the rhizobacteria isolated from sweet potato.

Bacterial strain	**Gram reaction (with 3%KOH)**	**Phosphatase* (on PKV) - 7 days -**	**Amylase* - 3 days -**	**Bacterial strain**	**Gram reaction (with 3%KOH)**	**Phosphatase* (on PKV) - 7 days -**	**Amylase* - 3 days -**
Dj1	G +	–	–	Dj16	G +	–	+ 2mm
Dj2	G +	–	–	Dj17	G –	+ 4mm	–
Dj3	G +	+ 1mm	+ 15mm	Dj18	G +	–	–
Dj4	G +	–	–	Dj19	G –	+ 2mm	–
Dj5	G +	–	–	Dj20	G +	+ 2mm	–
Dj6	G +	+ 1mm	+ 5mm	Dj21	G –	+ 2mm	–
Dj7	G +	–	–	Dj22	G –	+ 2mm	–
Dj8	G +	–	–	Dj23	G +	–	–
Dj9	G –	+ 2mm	+ 2mm	Dj24	G –	–	+ 5mm
Dj10	G –	–	–	Dj25	G +	+ 1mm	+ 12mm
Dj11	G –	+ 2mm	+ 1mm	Dj26	G –	+ 1mm	–
Dj12	G –	–	–	Dj27	G +	+ 1mm	+ 3mm
Dj13	G +	–	+ 1mm	Dj28	G –	+ 1mm	+ 1mm
Dj14	G –	–	+ 19mm	Dj29	G +	+ 3mm	+ 1mm
Dj15	G –	–	–	Dj30	G +	+ 1mm	+ 1mm

Legend: ”G“ gram reaction, ”–“ negative reaction, ”+“ positive reaction, * clear zone width induced by the enzyme.

Twenty-two of the isolated bacterial strains were also examined for their ability to produce auxin phytohormone. Indole 3-acetic acid (IAA) was quantified in cultures obtained in LB broth and LB supplemented with tryptophan, as precursor of IAA phytohormone. Among the tested bacterial isolates, four of them, designated as Dj 17, Dj 19, Dj 21 and Dj 30 produced high amounts of IAA (Table 3), therefore suggesting their potential in plant growth stimulation. These strains were able to synthesize 42.76 to 57.6 µg IAA/ml in LB broth and 90.14 to 98.08 µg IAA/ml in LB with tryptophan. According to these results, our strains were superior to the plant growth promotion strains isolated by ISLAM et al. (2016) where the maximum concentration of IAA was 51.28 µg IAA/ml in the culture medium supplemented with 2 mg/ml L-tryptophan. These results suggest that some of these isolates possess a number of traits associated with plant growth promotion.

To evaluate the biocontrol potential of the isolated strains, we analyzed *in vitro* some of the mechanism involved in direct competition against plant pathogens. Thus, we identified sixteen bacterial isolates with both swimming and swarming motility, which can easily colonize the rhizosphere and compete the pathogens of this niche. Enzymes involved in fungal cell wall degradation were also screened, but only nine strains (Dj3, Dj6, Dj9, Dj11, Dj14, Dj18, Dj22, Dj24, and Dj29) revealed cellulase activity and three strains (Dj3, Dj6 and Dj24) revealed chitinase production (Table 3).

Table 3. Production of IAA phytohormone by the rhizobacterial strains isolated from sweet potato.

Bacterial strain	**IAA (µg/ml)**		**Bacterial strain**	**IAA (µg/ml)**	
	LB	**LB with 5mM tryptophan**		**LB**	**LB with 5mM tryptophan**
Dj1	1.7	3.79	Dj18	19.82	27.4
Dj3	8.37	14.05	**Dj19**	**57.24**	**90.14**
Dj6	9.61	15.42	Dj20	17.55	34.62
Dj7	0.72	8.24	**Dj21**	**57.6**	**92.91**
Dj8	8.24	24.63	Dj22	16.8	54.66
Dj9	1.44	3.4	Dj24	10.05	25.1
Dj10	9.8	16.73	Dj25	1.76	7.19
Dj11	1.5	4.05	Dj27	2.61	37.65
Dj14	2.22	5.42	Dj28	6.01	36.5
Dj16	5.1	6.99	Dj29	17.98	58.86
Dj17	**49.62**	**97.34**	**Dj30**	**42.76**	**98.08**

The rhizobacterial strains were also tested for acetoin production, using Voges-Proskauer test (Table 3). Six of the analyzed strains (Dj3, Dj17, Dj19, Dj21, Dj23, Dj25) revealed clear acetoin production, however for other four strains (Dj6, Dj18, Dj20 and Dj24) this volatile compound was not always detected (in one of the three replicates). Acetoin (also known as 3-hydroxy-2-butanone), along with other microbial volatile compounds, such as 2,3-butanediol, produced by plant beneficial rhizobacteria, were shown to activate induced systemic resistance in the model plant *Arabidopsis thaliana* (RYU et al., 2003; RUDRAPPA et al., 2010).

Table 4. Biochemical analysis of the rhizobacteria isolated from sweet potato.

Bacterial strain	**Motility**		**Protease activity* (after 3 days)**			**Cellulase* (5 days)**	**Chitinase (5 days)**	**Acetoin (3 days)**
	Swimming	Swarming	Milk casein	Pure casein	Gelatine			
Dj1	+	+	–	–	+ 0.5cm	–	–	–
Dj2	+	–	+ 0.5cm	–	+ 1cm	–	N.A.	–
Dj3	+	+	+ 2cm	+ 1.2cm	+ 2.4cm	+ 0.6cm	+	+
Dj4	–	–	+ 0.4mm	–	+ 1.9 cm	–	N.A.	–
Dj5	–	+	+ 0.1cm	–	+ 0.6cm	–	N.A.	N.A.
Dj6	+	+	+ 2.2cm	+ 1.9cm	+ 2.4cm	+ 1cm	+	±
Dj7	–	+	+ 0.1cm	–	+ 0.9cm	–	–	–
Dj8	+	–	+ 0.1cm	–	–	–	–	–
Dj9	+	–	+ 0.7cm	–	+ 1.3cm	+ 1.2cm	–	–
Dj10	–	+	+ 1.4cm	+ 1.4cm	+ 1.4cm	–	–	–
Dj11	–	–	+ 0.1cm	–	–	+ 0.4cm	–	N.A.
Dj12	+	–	N.A.	–	–	–	N.A.	N.A.
Dj13	–	–	+ 0.5cm	–	+ 1.6cm	–	N.A.	–
Dj14	+	+	–	–	+ 1.7cm	+ 1.7cm	–	–
Dj15	–	–	–	–	+ 0.4cm	–	N.A.	–
Dj16	+	–	+ 0.2cm	+ 0.8cm	+ 1cm	–	–	–
Dj17	+	+	+ 0.5cm	–	+ 0.5cm	–	–	+
Dj18	+	+	+ 0.9cm	+ 1.4cm	+ 2.7cm	+ 1.9cm	–	±
Dj19	+	+	–	–	+ 0.7cm	–	–	+
Dj20	+	+	+ 0.6cm	+ 1.4cm	+ 2.2cm	–	–	±
Dj21	+	+	+ 0.2cm	–	+ 1cm	–	–	+
Dj22	+	+	+ 1.5cm	+ 1.5cm	+ 3cm	+ 0.9cm	–	–
Dj23	+	+	–	–	+ 0.5cm	–	N.A.	+
Dj24	+	+	+ 2.1cm	+ 1.8cm	+ 3.1cm	+ 1cm	+	±
Dj25	+	+	N.A.	+ 1.5cm	+ 2cm	–	–	+
Dj26	+	+	N.A.	–	–	–	N.A.	–
Dj27	+	+	N.A.	+ 0.6cm	+ 1.5cm	–	–	–
Dj28	+	+	N.A.	+ 0.9cm	+ 0.6cm	–	–	N.A.
Dj29	–	–	N.A.	+ 1.5cm	+ 1.8cm	+ 0.8cm	–	–
Dj30	–	–	N.A.	–	+ 0.3cm	–	–	–

Legend: ”–“ negative reaction, ”+“ positive reaction, ”N.A.“ not available, * clear zone width induced by the enzyme.

Although most of the isolated strains (~80%) revealed biocontrol and plant growth promoting traits, only five strains revealed *in vitro* antifungal activity against Fusarium stem rot of sweet potato (Dj3, Dj6, Dj17, Dj19, and Dj24). The strains Dj17 and Dj19 delayed the fungal growth in the first 5 days of co-cultivation, and Dj24 strain limited de fungal development at the vicinity of the bacterial colony. The best antifungal activity was revealed by the strains Dj 3 and Dj6, which expressed 62.35% and 61.18% inhibitory efficacy against the pathogen. Moreover, Dj 3 imposed a clear inhibitory zone of 4mm, where the pathogen was not able to colonize the growth substrate (Fig. 6b). At contrary, Dj6 strain inhibited the spread of the pathogen on the nutrient medium by colonizing the substrate with the bacterial colony (Fig. 6). The microscopic analysis of the interaction zones, between the rhizobacteria and the fungal pathogen, revealed that bacterial motility and competition for nutrients and niche are the basic mechanisms involved in the antifungal activity of Dj6 strain (Fig. 6c). Although Dj3 strain expressed similar antifungal potential *in vitro*, compared to Dj6 strain, the mechanisms of interaction were quite different. The bacterial growth did not migrate near the fungal mycelia, however some extra cellular compounds were released from the microorganism and visibly affected the terminal endings of the hyphae. Several swelling of the *Fusarium* hyphae and cell membranes destruction were seen under the microscope, probable due to osmotic stress caused by bacterial antifungal metabolites excreted by the bacterial strain Dj3 (Fig. 6a).

Among the isolated rhizobacteria only 4 of them were selected for Biolog GEN III identification, Dj3 due to its antifungal metabolites production, Dj6 for its inhibitory action against *Fusarium* sp., Dj17 and Dj19 especially for their ability to produce phytohormones and solubilize phosphates.

The biochemical identification revealed that Dj3 and Dj6 are both *Bacillus* sp. strains, Dj3 strain being identified as *B. subtilis* ssp. *subtilis*, and Dj6 as *B. subtilis*/ *mojavensis.* These two species are omnipresent bacteria in the environment, commonly found in soil and rhizosphere, many strains being reported as biocontrol agents and formulated as plant protection products. The other two strains, detected as Gram negative bacteria, were identified as *Enterobacter* sp. (Dj17) and *Pantoea agglomerans* (Dj19). Although *Enterobacter* sp. are included on the list of opportunistic human pathogens, some studies showed that it could be used as plant growth regulator (GEORGIEVA, 2003).

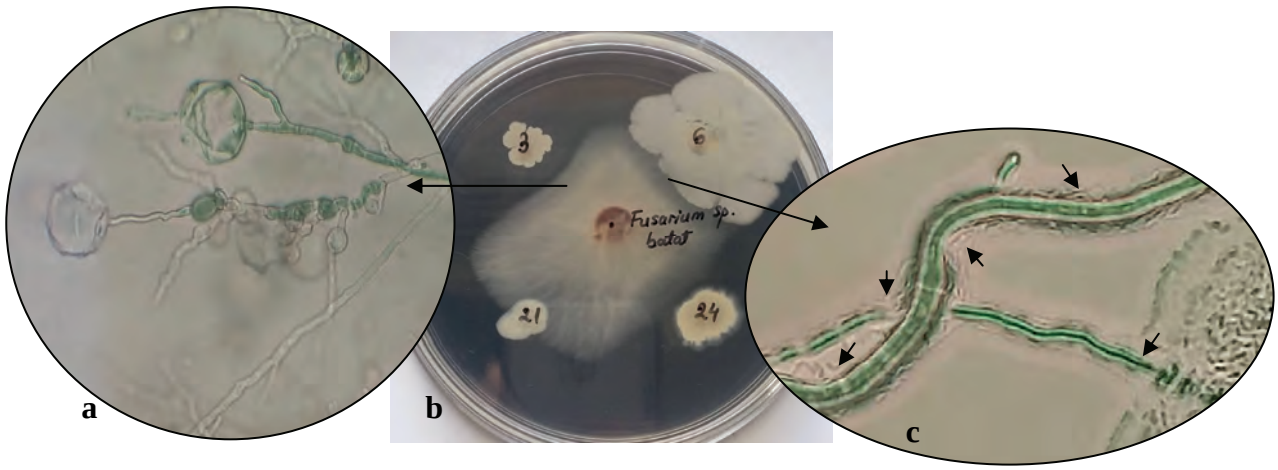

Figure 6. Bacterial inhibitory activities against *Fusarium* stem rot of sweet potato. **a.** swelling of the fungal cell walls caused by Dj3 bacterial metabolites, **b.** *in vitro* antagonistic activity of some beneficial rhizobacteria against *Fusarium* sp., and **c.** *Fusarium* hyphae colonized by Dj6 bacterial cells (originals).

Pantoea agglomerans however, was formerly known as *Enterobacter agglomerans* or *Erwinia herbicola*. This species is commonly associated with plant tissues, but it can also be found in animal and human faeces. Although, certain *P. agglomerans* strains were previously used as biocontrol agents, for the suppression of plant diseases, due to the biosafety concerns this microorganism could not be commercially registered for plant protection purposes (BONATERRA et. al, 2014). As *Bacillus subtilis* is worldwide accepted as a plant beneficial bacterium with various biocontrol mechanisms, Dj3 and Dj6 strains could be further used as potential biocontrol agents (SICUIA et al., 2015).

CONCLUSIONS

One case of *Fusarium* stem rot was detected in 2016, infecting the Korean variety Hayanmi growth in the sandy soils of southern Oltenia (Romania). The pathogen was identified based on its henotypic and biochemical characteristics with the Biolog system for filamentous fungi, but also based on the microscopic aspect and colony morphology.

For the biological control of this pathogen, thirty bacterial strains were isolated from the rhizosphere of healthy sweet potato, grown in the sandy soils of CCDCPN Dăbuleni. But only two of these isolates showed significant antagonistic activity against Fusarium stem rot. These strains were identified as *Bacillus subtilis* ssp. *subtilis* Dj3 and *Bacillus subtilis*/*mojavensis* Dj6. They expressed 61% to 62% *in vitro* inhibitory activity against Fusarium sp., and showed several lytic enzymes production, high motility and plant growth promoting activity, as mechanisms involved in plant protection against the fungal pathogenic attack.

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