ENDOPHYTES ACCESS PATHWAYS IN *Momordica charantia* L.

BOIU-SICUIA Oana-Alina, CORNEA Călina Petruța

Abstract. Endophytes are plant-associated microorganisms adapted to live inside plant tissues without causing harms to their hosts. Genomic studies showed that plant tissues, especially roots, are colonized by a wide spectrum of endophytic bacteria. Symbiotic associations are mediated by complex molecules released both by the plant and microorganisms. Such natural mechanism is already known in the endophytic association of rhizobia with compatible leguminous plants. Although host specificity for rhizobia is a known aspect, in nature, particular cases can be found. Indirect endophytic association can be possible when third parties are involved. Particular endopyte colonization can be found among symbiotic rhizobial-like bacteria and atypical host plants. This paper presents a particular case of endophytic association between bacteria of the genus *Ensifer* and the roots of *Momordica charantia* plants. The access of these bacteria to the plant is considered to be mediated by the attack of bitter mellon caused by the root-knot nematode.

Keywords: endophyte, *Ensifer* sp., bitter melon, root-knot nematode.

Rezumat. Căi de acces pentru endofiți în *Momordica charantia* L. Microorganismele endofite sunt adaptate să se dezvolte în interiorul țesuturilor vegetale fără să producă simptome plantelor gazdă. Studiile genomice au demonstrat că organele plantelor, în special rădăcinile sunt colonizate de un spectru larg de bacterii endofite. Asocierile simbiote sunt mediate de molecule complexe eliberate atât de către plante, cât și de către microorganisme. Astfel de mecanisme naturale de comunicare sunt cunoscute în rândul asocierilor endofite dintre rizobii și plantele leguminoase. Deși este cunoscut faptul că rizobiile prezintă specificitate pentru anumite plante gazdă, în natură pot fi întâlnite unele excepții. Colonizarea plantelor cu microorganisme endofite poate avea loc indirect prin implicarea unor intermediari. Astfel, rizobiile pot coloniza endofit și alte specii de plante gazdă, comparativ asocierilor simbionte cu plantele leguminoase. În lucrarea de față este prezentat un caz particular de asociere endofită între bacterii din genul *Ensifer* și rădăcinile plantelor de *Momordica charantia*. Accesul acestor bacterii în plantă este considerat a fi mediat de atacul rădăcinilor de castravete amar cauzat de nematozi galicoli.

Cuvinte cheie: endofiți, *Ensifer* sp., castravete amar, nematozi galicoli.

INTRODUCTION

Momordica charantia L., also known as bitter melon, is originally from South-Oriental Asia. It is mostly grown and consumed in Asian and South American countries (PEREZ et al., 2019). In Europe it is grown as medicinal plants due to its hypoglycaemic properties and beneficial attributes given by its health promoting compounds, such as ascorbic acid, polyphenols and triterpenoids. However, bitter melon extracts are mostly used for their antidiabetic effect (SĂRĂNDAN et al., 2010; CHAUBEY et al., 2019; CHOKKI et al., 2020).

Studies on endophyte communities in medicinal plants have shown that some associated microorganisms can increase health-beneficial compounds in their host plant (JIA et al., 2016; MAGGINI et al., 2017; 2019). However, most studies on the endophyte influence on plants are focused on growth promotion and increase resistance to biotic and abiotic stress. Beneficial effects on the physical parameter of bitter melon (*M. charantia*) were also reported after plant inoculation with *Azomonas agilis* and *Ensifer adhaerens* endophytic strains (MUSHTAQ et al., 2018).

This study aims to present an endophytic association between *Ensifer* sp. bacteria and bitter melon plants, mediated by root-knot nematodes attack.

MATERIALS AND METHODS

Plant examination. *Momordica charantia* with heavy root galling (Fig. 1) were brought for laboratory analysis. Roots were washed with tap water using a smooth brush to remove the extra soil. Gall pieces were detached, sectioned and immersed in warm water. After 15 to 20 minutes biological samples were collected and analysed under the stereomicroscope and optical microscope, using reflected and transmitted light.

Bacteria isolation protocol. Bitter melon root samples were washed and the surface was disinfected with 4% sodium hypochlorite. After rinsing, plant material was grinded in phosphate saline buffer and let to infuse for 20 minutes. Aliquots were plated on MG-Te medium (OPHEL & KERR, 1990). Bacteria were then purified through the streak plate method on MG, and maintained in pure culture on Yeast extract Mannitol Agar (YMA). Glycerol stocks were also prepared and stored at -20°C.

Bacteria phenotypic identification. Based on their physiological profile, bacteria were identified using the Biolog GEN III technique and standard IF-A protocol. The procedure concerned bacterial cultivation on YMA to obtain fresh isolated colonies. The biomass was than suspended in Biolog A type inoculation fluid (IF-A) up to 97% turbidity, at 590 nm wavelength. The resulting inoculum was distributed 100µl/well in Biolog GEN III microplates. Overnight cultures, obtained at 33°C, were subjected to the semi-automated Biolog Microstation Plate Reader in order to analyse their physiological profiles and reveal biochemical identification.

Figure 1. Root galls in *M. charantia* (original).

RESULTS AND DISCUSSIONS

Root-knot nematode determination. Visual and stereomicroscope analysis suggested that heavy root galling of bitter melon plants is caused by some root nematodes attack (Fig. 2).

Figure 2. Root-knot nematode galls in *M. charantia* (original).

However, root samples were immersed in warm water in order to confirm the presence of such pests into plant tissue. After several minutes, numerous silky white swollen bodies were seen inside and out from the root galls (Fig. 3a). These were transferred on microscopic slides and further analysed. Nematode females, with pear-shaped body and no posterior protuberance, were seen (Fig. 3b).

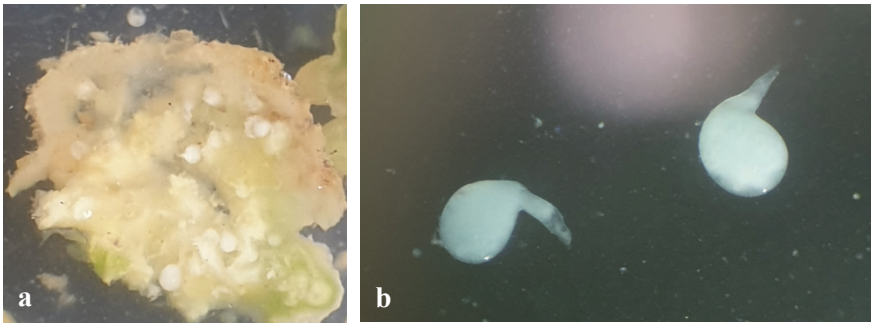

Figure 3. Female nematodes (original)
a. Female nematodes in the root tissue, b. Pear shape female nematodes.

In the immersion water, nematode eggs in various developmental stages were seen under the microscope (Fig. 4a; b). Juvenile nematodes, including J2 infective stages were also noticed (Fig. 4c). Sharp stylet with basal knobs was detected to such pests, confirming parasitic nematodes.

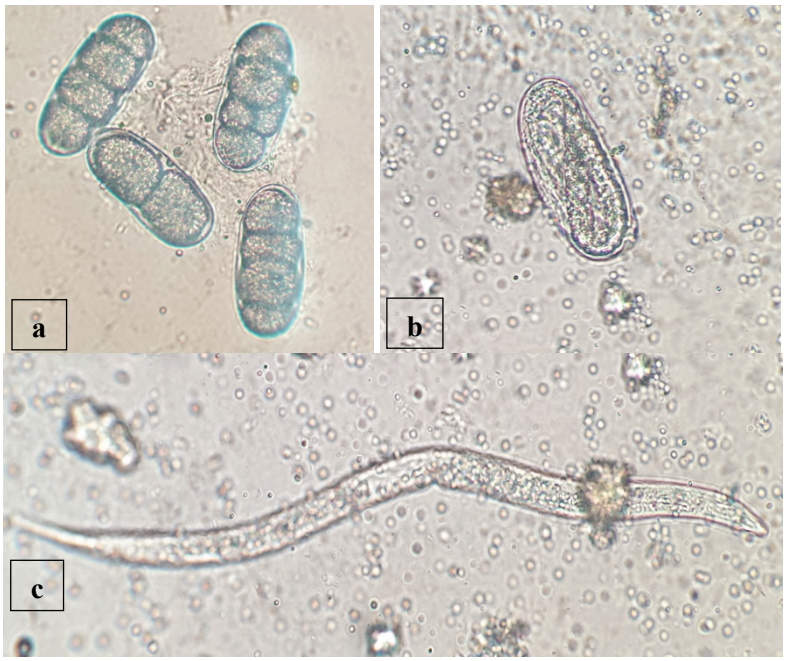

Figure 4. Nematodes in different growth stages:
a. egg stage; b. first stage juvenile inside egg; c. second stage juvenile hatch from egg.

These root injuries caused by nematodes are increasing the plants' possibility to contact endophytic microorganisms from the soil and rhizosphere (BOIU-SICUIA & CORNEA, 2020). Similar aspects are mentioned by various research groups (HARDOIM et al., 2015; KANDEL et al., 2017; MARAG & SUMAN, 2018), sustaining such access pathways for endophytic microbial colonizers. However, wounds and injuries are potential entry sites for various microorganisms, beneficial or neutral, and even detrimental, with common or accidental endophytic behaviour.

Isolated bacteria. The microbial growth obtained on MG-Te agar revealed dark, smooth, glittery colonies (Fig. 5a). Purified cultures on YEM revealed round to irregular colonies, raised from the agar surface, with a smooth margin, white biomass (Fig. 5b) and slimy, moist texture. A similar morphology, with small differences in terms of colony size, was noticed within the isolates from primary (MC rad1) and secondary roots (MC rad2) and plant crown (MCc).

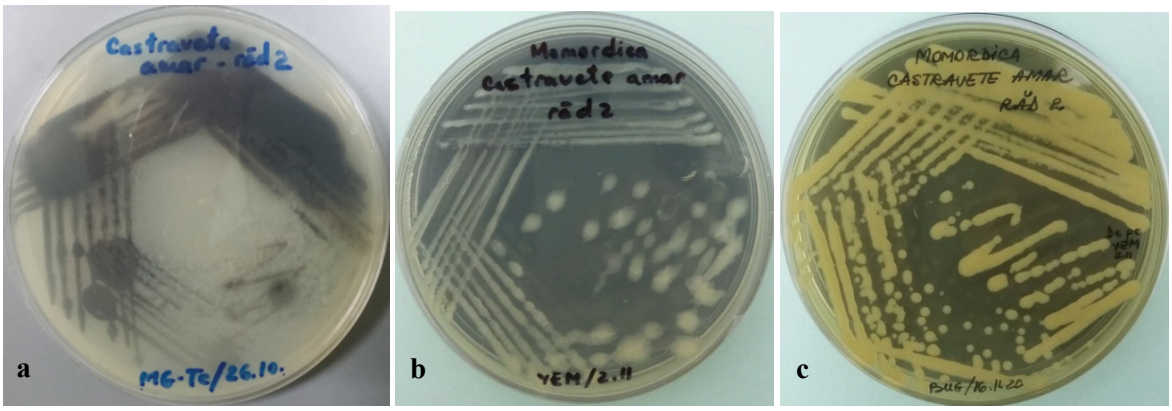

Figure 5. Endophyte bacteria obtained from secondary roots of nematode attacked *M.charantia.*
Bacterial growth on MG-Te (a), on YEM agar (b) and BUG medium (c)

A comparable bacterial morphology can be seen in some rhizobia cultures. However, our strains were able to grow on BUG medium. On this substrate the colonies were circular, smooth and convex (figure 4c).

Bacteria biochemical characterization. Bacterial physiological profiles were obtained due to their metabolic ability to use certain carbon sources and grow in the presence of certain salt concentration, pH values or chemical inhibitors.

Regarding the ability to use potential nutritive sources, such as sugars, amino acids, hexose acids, carboxylic acids, esters and fatty acids, the MC rad2 strain was able to metabolise 60 carbon sources of the 71 tested. Nutrients improper for bacterial growth were: 3-methyl glucose, D-fructose-6-PO_4, D-serine, D-saccharic acid, methyl pyruvate, tween 40, hydroxy-butyric acids, α-keto-butyric acid, propionic and formic acids (Table 1).

Table 1. Endophytes sugar metabolism.

Sugar substrates and derivatives	Bacterial strains		
	MC rad1	MC rad2	MCc
Dextrin	+/–	+	+/–
D-Maltose	+	+	+
D-Trehalose	+	+	+
D-Cellobiose	+/–	+	+
Gentiobiose	+	+	+
Sucrose	+	+	+
D-Turanose	+	+	+
Stachyose	–	+/–	–
D-Raffinose	–	+	–
α-D-Lactose	+/–	+	–
D-Melibiose	–	+	–
β-Methyl-D-Glucoside	–	+	+/–
D-Salicin	–	+/–	+/–
N-Acetyl-D-Glucosamine	+	+	+
N-Acetyl- β-D-Mannosamine	+	+	+
N-Acetyl-D-Galactosamine	–	+	–
N-Acetyl Neuraminic Acid	–	+/–	+/–
α-D-Glucose	+	+	+
D-Mannose	+	+	+
D-Fructose	+	+	+
D-Galactose	+	+	+
3-Methyl Glucose	–	–	–
D-Fucose	+/–	+	+
L-Fucose	+	+	+
L-Rhamnose	+/–	+	+
Inosine	–	+/–	–
Legend: + bacterial growth, +/– borderline, – no bacterial growth			

Regarding the tolerance to potential inhibitors, MC rad2 revealed the possibility to grow in the presence of 1% sodium lactate, lincomycin, niaproof 4, nalidixic acid, aztreonam, and was resistant to troleandomycin, rifamycin SV and potassium tellurite (Table 2).

Table 2. Endophytes chemical inhibitors.

Inhibitors	Bacterial strains		
	MC rad1	MC rad2	MCc
Sodium lactate	+	+/–	+/–
Fusidic acid	–	–	–
Troleandomycin	+	+	–
Rifamycin SV	+	+	+/–
Minocycline	–	–	–
Lincomycin	+	+/–	+/–
Guanidine hydrochloride	+	–	–
Niaproof 4	+/–	+/–	–
Vancomycin	–	–	–
Nalidixic acid	+/–	+/–	–
Lithium chloride	+/–	–	–
Potassium tellurite	+	+	+/–
Aztreonam	+	+/–	–
Sodium butyrate	+/–	–	–
Sodium bromate	–	–	–
Legend: + resistant, +/– tolerant, – sensitive			

MC rad1 and MCc strains showed many similarities. However, the first strain could metabolize only 54.93% carbon sources of the 71 tested, and compared to MC rad2 was resistant to guanidine hydrochloride and could tolerate lithium chloride and sodium butyrate. The other strain, MCc, metabolized 63.38% carbon sources of 71 tested, and compared to MC rad2 was sensitive to troleandomycin, niaproof 4, nalidixic acid and aztreonam.

Bacterial identification. Subjected to Biolog GEN III identification, only the strain isolated from secondary roots was attributed to a taxon. The identification was reliable only at the genus level, where the MC rad2 strain was affiliated to *Ensifer* sp. The similarity with *Ensifer meliloti* was 51.6%, too low for a species-level identification. The other strains revealed too different pattern from the microbial database. Although, according to most probable species, they belong to the rhizobia group of bacteria, being similar to *Phyllobacterium* sp.

Endophyte access pathway. Studies on endophytic bacteria described various *Ensifer* and *Phyllobacterium* species capable to colonise internal plant tissue (MANTELIN et al., 2006; FLORES-FELIX et al., 2013; MESA et al., 2017). However, the *Ensifer* genus is harbouring both symbiotic and non-symbiotic species (FAGORZI et al., 2020). In *Momordica charantia*, the endophyte inoculation of *Ensifer adhaerens* revealed plant beneficial effects on the physical

parameter (MUSHTAQ et al., 2018). However, to our knowledge, this is the first report of such endophyte bacteria collected from bitter melon plants. Still, due to the root damage caused by nematode attack, it is debatable whether such bacteria are able to establish an endophytic relationship with *M. charantia* plants or such endosphere colonisation was mediated by the parasitic nematodes that caused root cracks.

CONCLUSIONS

Endophytic colonization is initiated after a complex communication between plants and adjacent microbial communities. Chemical signals released by the plants change the behaviour of surrounding microbes, also affecting neighbouring life forms. The endophytic lifestyle can be achieved by soil-borne, rhizosphere, air-transported or epiphytic microorganisms that find a way to enter inside plants by active or passive manners. The access pathways of soil and rhizosphere microorganisms into plants could be through natural opened sites such as root cracks, degraded root hairs, natural injuries or injuries caused by third parties and wounds caused by abiotic or biotic factors. This study is presenting a case of nematode attack contributing to microbial admission and endophytic colonization of *Momordica charantia* roots.

ACKNOWLEDGMENTS

We are grateful to Maria Dinu for sharing her knowledge regarding samples preparation for nematodes analysis.

REFERENCES

BOIU-SICUIA OANA-ALINA & CORNEA CĂLINA PETRUȚA. 2020. Bacterial endophytes improving plant growth. *AgroLife Scientific Journal*. University of Agronomic Sciences and Veterinary Medicine of Bucharest. **9**(2): 56-70.

CHAUBEY PRAMILA, SUVARNA V., SANGAVE P. C., SINGH A. K. 2019. Nutritional management of diabetes – A critical review. Chapter 19 In: *Bioactive Food as Dietary Interventions for Diabetes* (Second Edition). Watson Ronald Ross, Preedy Victor R. (eds). Academic Press. London: 289-308.

CHOKKI MICHAELLE, CUDĂLBEANU MIHAELA, ZONGO CHEIKNA, DAH-NOUVLESSOUNON D., GHINEA IOANA OTILIA, FURDUI BIANCA, RACLEA R., SAVADOGO ALY, BABA-MOUSSA LAMINE, AVAMESCU S. M., DINICA R. M., BABA-MOUSSA F. 2020. Exploring antioxidant and enzymes (α-amylase and β-glucosidase) inhibitory activity of *Morinda lucida* and *Momordica charantia* leaves from Benin. *Foods*. MDPI Publisher. London. **9**: 434-439.

FAGORZI CAMILLA, ILIE A., DECOROSI FRANCESCA, CANGIOLI LISA, VITI C., MENGONI M., DICENZO G.C. 2020. Symbiotic and nonsymbiotic members of the genus *Ensifer* (syn. *Sinorhizobium*) are separated into two clades based on comparative genomics and high-throughput phenotyping. *Genome Biology and Evolution*. Oxford Academic. London. **12**(12): 2521-2534.

FLORES-FELIX J. D., CARRO LORENA, VELAZQUEZ E., VALVERDE A., CERDA-CASTILLO EUGENIA, GARCIA-FRAILE PAULA, RIVAS R. 2013. *Phyllobacterium endophyticum* sp. nov., isolated from nodules of *Phaseolus vulgaris*. *International Journal of Systematic and Evolutionary Microbiology*. Microbiology Society. London. **63:** 821-826.

HARDOIM P. R., VAN OVERBEEK L. S., BERG GABRIELE, PIRTTILÄ ANNA MARIA, COMPANT S., CAMPISANO A., DÖRING M., SESSITSCH ANGELA. 2015. The hidden world within Plants: Ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiology and Molecular Biology Reviews*. American Society for Microbiology. New York. **79**: 293-320.

JIA M., CHEN L., XIN H.L., ZHENG C.J., RAHMAN K., HAN T., QIN L.P. 2016. A friendly relationship between endophytic fungi and medicinal plants: A systematic review. *Frontiers in Microbiology*. Frontiers. London. **7**: 906-912.

KANDEL S. L., JOUBERT P. M., DOTY SHARON L. 2017. Bacterial endophyte colonization and distribution within plants. *Microorganisms*. MDPI Press. London. **5**(4): 77-83.

MAGGINI VALENTINA, DE LEO MARINELLA, GRANCHI CARLOTTA, TUCCINARDI T., MENGONI A., GALLO EUGENIA ROSARIA, BIFFI S., FANI R., PISTELLI LUISA, FIRENZUOLI F., BOGANI PATRIZIA. 2019. The influence of *Echinacea purpurea* leaf microbiota on chicoric acid level. *Scientific reports*. Springer Nature Limited. Berlin. **9**: 10897-10903.

MAGGINI VALENTINA, DE LEO MARINELLA, MENGONI A., GALLO EUGENIA ROSARIA, MICELI ELISANGELA, BANDEIRA REIDEL ROSE VANESSA, BIFFI S., PISTELLI LUISA, FANI R., FIRENZUOLI F., BOGANI PATRIZIA. 2017. Plant-endophytes interaction influences the secondary metabolism in *Echinacea purpurea* (L.) Moench: an *in vitro* model. *Scientific reports*. Springer Nature Limited. London. **7**: 16924-16930.

MANTELIN SOPHIE, SAUX M. F., ZAKHIA F., BENA G., BONNEAU SOPHIE, JEDER H., DE LAJUDIE P., CLEYET-MAREL J. C. 2006. Emended description of the genus *Phyllobacterium* and description of four novel species associated with plant roots: *Phyllobacterium bourgognense* sp. nov., *Phyllobacterium ifriqiyense* sp. nov., *Phyllobacterium leguminum* sp. nov. and *Phyllobacterium brassicacearum* sp. nov. *International Journal of Systematic and Evolutionary Microbiology*. Microbiology Society. London. **56**: 827-839.

MARAG P. S. & SUMAN ARCHNA. 2018. Growth stage and tissue specific colonization of endophytic bacteria having plant growth promoting traits in hybrid and composite maize (*Zea mays* L.). *Microbiological Research*. Elsevier. Paris. **214**: 101-113.

MESA VICTORIA, NAVAZAS A., GONZÁLEZ-GIL R., GONZÁLEZ AIDA, WEYENS NELE, LAUGA BÉATRICE, GALLEGO, J. SÁNCHEZ J., PELÁEZ ANA ISABEL. 2017. Use of endophytic and rhizosphere bacteria to improve phytoremediation of arsenic-contaminated industrial soils by autochthonous *Betula celtiberica*. *Applied and Environmental Microbiology*. American Society for Microbiology. New York. **83**(8): 3411-3416.

MUSHTAQ S., SHAFIQ M., KHAN FAIZA SHAFIQUE, ASHRAF T., HAIDER, M. S. 2018. Effect of bacterial endophytes isolated from the citrus on the physical parameter of bitter gourd (*Momordica charantia* L.). *World Journal of Biology and Biotechnology*. Scientific Press Limited. London. **3**(2): 193-197.

OPHEL KATHY & KERR A. 1990. *Agrobacterium vitis* sp. nov. for strains of *Agrobacterium* biovar 3 from grapevines. *International Journal of Systematic Bacteriology*. International Union of Microbiological Societies. London. **40**(3): 236-241.

PEREZ J. L, JAYAPRAKASHA G. K, CROSBY K., PATIL B. S. 2019. Evaluation of bitter melon (*Momordica charantia*) cultivars grown in Texas and levels of various phytonutrients. *Journal of the Science of Food and Agriculture*. Wiley Press. London. **99**: 379-390.

SĂRĂNDAN H., BOTĂU DORICA, IANCULOV I., RADU FLORINA, RADA OLGA, MORAR D., SĂRĂNDAN M., ȘERB MARIA, ANGHEL ADELA. 2010. The hypoglicemic effect of *Momordica charantia* Linn in normal and alloxan induced diabetic rabbits. *Scientific Papers: Animal Science and Biotechnologies*. AGROPRINT Timișoara. **43**(1): 516-518.

Boiu-Sicuia Oana-Alina, Cornea Călina Petruța
University of Agronomical Sciences and Veterinary Medicine Bucharest, Faculty of Biotechnologies, 59 Mărăști Blvd, Bucharest, Romania.
E-mails: sicuia_oana@yahoo.com; cpcornea@yahoo.com

Boiu-Sicuia Oana-Alina
Research and Development Institute for Plant Protection, 8 Ion Ionescu de la Brad Blvd., Bucharest, Romania.
E-mail: sicuia_oana@yahoo.com

Received: April 14, 2021
Accepted: July 23, 2021