NARDI FUNDULEA, ROMANIA
www.incda-fundulea.ro; doi.org/10.59665/rar4057
ROMANIAN AGRICULTURAL RESEARCH, NO. 40, 2023
Print ISSN 1222-4227; Online ISSN 2067-5720

SELECTED PLANT PROTECTION *Bacillus* STRAINS INCREASE FOOD SAFENESS BY INHIBITING HUMAN PATHOGENIC BACTERIA

Radu Cristian Toma[1], Oana-Alina Boiu-Sicuia[1,2*], Filofteia Camelia Diguță[1], Matilda Ciucă[3], Florentina Matei[1], Călina Petruța Cornea[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Mărăşti Blvd, no. 59, 011464 Bucharest, Romania
[2]Research-Development Institute for Plant Protection, Ion Ionescu de la Brad Blvd, no. 8, 013813 Bucharest, Romania
[3]National Agricultural Research and Development Institute Fundulea, 915200 Fundulea, Călăraşi County, Romania
[*]Corresponding author. E-mail: sicuia_oana@yahoo.com

ABSTRACT

Food illnesses can occur due to the presence of human pathogen contaminants in fresh farm products. Herbs, vegetables and fruits, especially from organic agriculture, are highly exposed to animal and human pathogens. However, safe microbial antagonists, approved for plant protection, could be a solution to prevent this health risk to occur. The aim of this study is to reveal several beneficial bacterial strains reducing the prevalence of human and animal pathogens. Tested beneficial strains were previously described as promising biocontrol agents against soilborne pathogens of field crops and vegetables. Moreover, their endophyte adaptation, ensures an intimate relation with their plant hosts. Therefore, within this study we analyzed the inhibitory activity of seven biocontrol endophytes against 24 reference bacterial strains, of which 19 important human and animal pathogens. Some of the tested beneficial strains revealed antibacterial activity against a wide spectrum of pathogens, such as: *Bacillus cereus, Enterococcus faecalis, Escherichia coli, Listeria ivanovii, L. monocytogenes, Rhodococcus equi, Salmonella enterica, S. typhimurium, Staphylococcus aureus, S. epidermidis* and *Streptococcus pyogenes*. Due to their antagonistic activity, the beneficial strains were studied through molecular techniques to reveal their functional genes involved in antimicrobial compounds synthesis. Genes encoding for iturin A, surfactin, bacilysin, bacillomycin and bacillaene were found in these biocontrol strains. Therefore, we could consider such beneficial strains as promising candidates for plant protection and human safety.

Keywords: biocontrol endophytes, antagonism, pathogenic bacteria, functional genes.

INTRODUCTION

Today demand for low toxicity and eco-friendly agricultural inputs increased the trust in alternative control methods to chemicals (Brumă et al., 2021). Although for plant nutrition manure and organic composts are good to stimulate plant growth and development (Cirebea et al., 2020), as they increase soil fertility and improve soil structure, unfortunately sometime it could bring various pathogenic load. Studies have showed that using manure or compost, less than one-year old, increases 19 times the prevalence of *E. coli*, compared to older aged materials (Mukherjee et al., 2004).

Herbs and vegetables, as well as some fruits, especially from organic agriculture, are highly exposed to animal and human pathogens contamination. For sure contamination can also occur during harvesting or while post-harvest handling (Alsanius et al., 2016). However, there are several cases explicitly linked to the consumption of organic plant products (Meerburg and Borgsteede, 2011). In 1992, a child lost his life due to an *E. coli* O157:H7 infection counteract while eating improper washed vegetables obtained from manure fertilized garden (Cieslak et al., 1993). In 1995, severe gastroenteritis and cases of haemolytic uraemic syndrome were attributed to *Citrobacter freundii* in a nursery school, were sandwiches with green butter, containing contaminated organic parsley, were given to the children (Tschäpe et al., 1995). Later on, in 2000, a comparative study was carried out in organic and conventionally produced lettuce and alfalfa sprouts, in order to quantify

Received 30 November 2022; accepted 18 January 2023. First Online: January, 2023. DII 2067-5720 RAR 2023-114

the presence of important human pathogens. Higher load of *E. coli* (10^6 cfu/g) was found in organic lettuce spring mix (8 of 48 samples), compared to conventional spring mix (4 of 48 samples). *Salmonella* spp. was found in organic produced alfalfa sprouts in 7.7% cases (3 of 39 samples), while the conventional sprouts were free of pathogenic bacteria (Doyle, 2000).

There are also studies were there were no significant differences in the microbial load when analyzing different varieties of organic and conventional vegetables (Maffei et al., 2013). These showing that hygiene is very important in each production system.

Only a few studies were made to evaluate human pathogens presence in cereals. In Australia, Europe and United States, the prevalence of *B. cereus*, *E. coli*, and *Salmonella* spp. in wheat and flour is at low levels (Berghofer et al., 2003). However, in African countries, various cases of enteropathogenic *E. coli* were detected in maize and millet flours, in Côte d'Ivoire (Kouame N'zebo et al., 2017).

Unfortunately, important pathogens such as *Salmonella* spp., *L. monocytogenes* and *E. coli* could be present in organic fertilizers, such as manure (Johannessen et al., 2004). However, in the European Union, the new Regulation (EU) 2019/1009 on the fertilizing products available on the market provides safer organic fertilizers. Therefore, in certified organic agriculture, pathogens like *E. coli* or *Enterococcaceae* must not exceed 10^3 cfu in 1 g or 1 ml of organic fertilizer, while *Salmonella* spp. must be absent in 25 g or 25 ml of marketable product.

The European Union initiative, through the 'farm to fork' strategy, intend to facilitate the approval of beneficial microorganisms on the market, as plant protection products in order to reduce the dependence on synthetic chemicals. Therefore, starting from November 21st 2022 new regulations on the approval of microorganisms as active substances [Commission Regulation (EU) 2022/1438 amending Annex II to Regulation (EC) No 1107/2009], specific data requirements for such active substances [Commission Regulation (EU) 2022/1439, amending Regulation (EU) No 283/2013] and commercial plant protection products [Commission Regulation (EU) 2022/1440, amending Regulation (EU) No 284/2013], as well as uniform principles for their evaluation and authorization [Commission Regulation (EU) 2022/1441, amending Regulation (EU) No 546/2011] are now applicable. However, several microbial based products are already available on the market (Abuhena et al., 2022), previously approved as plant protection products based on the same legislation as for synthetic pesticides. But new strains of microbial antagonists will be easier released on the market and used to control plant pathogens. Among plant beneficial microorganisms there could be found promising bio-fertilizing, bio-stimulant and biocontrol strains within *Bacillus subtilis* group (Sicuia et al., 2015).

Bacillus spp. are promising microorganisms for agriculture. They are able to produce various growth promoting and biocontrol metabolites (Ek-Ramos et al., 2019; Boiu-Sicuia and Cornea, 2020, 2021). Their enzymatic activity is useful not only in agriculture and environmental applications but also in several industries (Su et al., 2020). They are highly versatile and can adapt to various environmental conditions. Moreover, their sporulation ability makes them reliable for long term storage and extreme conditions, without affecting to much their viability (Kefi et al., 2015). An important aspect about *B. subtilis* is the fact that the European Food Safety Authority (EFSA) recognized it as qualified presumption of safety and accept it as zootechnical additive and plant protection product (Koutsoumanis et al., 2020; Spears et al., 2021). The use of *B. subtilis* and related bacteria as bio-based agro-inoculants is accepted also in organic farming (Ostroukhova et al., 2022). Strains of *Bacillus* sp., isolated from soil or fermented foods, demonstrated good antibacterial activity against human pathogens such as *B. cereus*, *E. coli* O157:H7, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *S. typhimurium* and *Staphylococcus aureus* (Avcı et al., 2016).

Considering this, the aim of this study is to reveal several plant beneficial bacterial strains that can be used to promote plant

health and nutrition while reducing the prevalence of human and animal pathogens.

MATERIAL AND METHODS

Beneficial bacteria and growth conditions

Seven endophytic bacterial strains, isolated from various plant species grown in Romania, were used in this work (Table 1). These strains were previously selected for their plant beneficial characteristics (Boiu-Sicuia and Cornea, 2021), such as antagonism against various pathogenic fungi, or traits related to plant growth promotion.

Pathogenic bacteria and growth conditions

Various human and animal pathogenic bacteria were used in this study (Table 2). Both Gram positive and Gram negative, of 11 different genera, some of them antibiotic resistant strains. Beside pathogenic strains, commensals and non-pathogenic species, were also used as sensitive references for antibacterial tests. All strains used for bactericide testing were reference strains from the American Type Culture Collection (ATCC).

Endophytes were freshly grown on Luria Bertani (LB, Carl Roth GmbH + Co.KG) at 28°C. The submersed cultures were incubated under orbital shaking at 150 rpm. Cultures were stored in LB Broth with 25% glycerol at -20°C.

These bacteria were stored in Tryptic Soy Broth (TSB, Oxoid™) with 20% glycerol at -20°C. Cultures were refreshed on Tryptic Soy Agar (TSA, Oxoid™) and routinely cultured on TSB at 37°C before each trial.

Table 1. Sources of plant beneficial bacteria

Endophytic Strain	Isolation source	
	Host plant	Plant part
LT MYM 1	*Lavandula angustifolia*	lavender stem
LFF MYM 5	*Lavandula angustifolia*	lavender leaves and flowers
St 1T2	*Solanum tuberosum*	potato tuber
E1Pv	*Phaseolus vulgaris*	bean roots
BPVs2	*Phaseolus vulgaris*	bean seeds
BAHs1	*Arachis hypogaea*	peanut seeds
BTAs3	*Triticum aestivum* cv. Glosa	wheat kernels

Table 2. Pathogenic bacteria and other reference strains

Species	Type strain	Special characteristics
Gram positive bacteria		
Bacillus cereus	ATCC 11778	human and animal pathogen; it can cause foodborne illness and a wide range of opportunistic infections
B. subtilis	ATCC 6633[i]	non-pathogenic specie; antibiotic sensitive strain
Enterococcus faecalis	ATCC 29212	vancomycin-sensitive; could be found as opportunistic pathogen
Ent. Faecium	ATCC 6057[ii]	commensal bacterium which gain prominence as a nosocomial pathogen
Ent. Hirae	ATCC 10541	zoonotic pathogen
Listeria innocua	ATCC 33090[i]	non-pathogenic specie
L. ivanovii	ATCC 19119	infect ruminants, and is rarely reported as human pathogen
L. monocytogenes	ATCC 7644	human and animal pathogen responsible for food borne illness
	ATCC 13932	enteric and infectious disease
Rhodococcus equi	ATCC 6939	responsible for infections in multiple-hosts, animals and humans
Staphylococcus aureus subsp. *aureus*	ATCC 6538	human pathogenic specie
	ATCC 25923	human pathogenic specie
	ATCC 33592	gentamicin and methicillin resistant strain; responsible for a wide spectrum of clinical infections
	ATCC 43300	methicillin and oxacillin resistant strain; responsible for a wide spectrum of clinical infections
S. epidermidis	ATCC 12228	vancomycin sensitive strain; important human opportunistic pathogen of nosocomial infections
	ATCC 51625	methicillin-resistant strain; important human opportunistic pathogen of nosocomial infections
Streptococcus pyogenes	ATCC 19615	human pathogenic specie
Gram negative bacteria		
Citrobacter freundii	ATCC 43864	responsible for nosocomial infections and diarrheal infections; it become a multidrug resistant specie
Escherichia coli	ATCC 8739	faecal strain; known as a human and animal pathogen
	ATCC 25922	human and animal pathogen
Proteus hauseri	ATCC13315[ii]	commensal of the normal flora of human gastrointestinal tract, that can switch in opportunistic pathogen
Pseudomonas aeruginosa	ATCC 9027[i]	can cause animals and humans diseases; non-virulent strain
Salmonella enterica subsp. *enterica* serovar *Enteritidis*	ATCC13076	an enteric foodborne pathogen
S. typhimurium subsp. *enterica* serovar *Typhimurium*	ATCC 14028	an enteric foodborne pathogen; multiple antibiotic resistances.

Legend: [i]Non-pathogenic bacteria or non-virulent strain; [ii]Com mensal bacteria.

Bacterial identification procedure

Endophytic bacteria were identified based on Biolog technique, using the semi-automated GEN III system, according to the manufacturer guidelines. Therefore, fresh cultures were prepared on Biolog Universal Growth (BUG) media. From the fresh cultures, single colonies were suspended in Biolog type B inoculation fluid up to 97% turbidity, in 590 nm light. Obtained bacterial suspension was inoculated in Biolog GEN III microplate, using 100 µl/well. Plates were incubated at 33°C. The microbial identification was made after 24 and 48 h of incubation using the semi-automat Biolog MicroStation Plate Reader coupled to the Microbial Identification Databases for Biolog Systems. The GEN III redox chemistry reveals the physiological prophyll of the analyzed strain and compares it to other 1568 taxa. The microbial preferences to metabolize certain carbon sources and tolerance certain chemicals, reveals the unique pattern of the strain and enables the identification to genus or species level if there is a proper correlation with the systems' database.

Plate screening of bacterial antagonism

The spot diffusion assay was used to evaluate the antibacterial activity of endophytic strains against mentioned human and animal pathogenic

bacteria. Tests were performed in vitro, on TSA medium. Overnight grown pathogens were inoculated in the melted agar in 1:10 (v/v) ratio, using up to 0.3 OD cell suspension quantified at 600 nm. On top of the solidified pathogenic cultures tested endophytes were inoculated in spots, using 5 µl of 24 h old plant beneficial bacteria. Control plates were also prepared, were instead of the pathogen, antibiotic sensitive strains were used. Tests were performed in triplicate and incubated at 30°C. Plates were analyzed after 24 to 48 h of incubation and the antibacterial activity was evaluated by measuring the inhibitory hallo revealed around the endophytic strains. Pathogens were considered sensitive to the endophytes if the inhibitory hallo were completely clear, while tolerant pathogens were considered those which revealed a tern growth in the presence of the antagonistic strain.

Antibacterial functional genes and PCR conditions

The presence of certain functional genes involved in antibacterial compounds synthesis was carried out by polymerase chain reaction (PCR).

Certain primers pairs were used in this study to reveal genes encoding for antimicrobial compounds (Table 3). The PCR mix was performed in 25 µl reaction volume containing 1X Buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs (ThermoScientific LSG), 0.5 µM of each primer (Alpha Scientific Solutions), 0.25 U of MangoTaq DNA Polymerase (BioLine) and 20 ng of template DNA. The DNA was purified using the ZR Fungal/Bacterial MiniPrep kit (Zymo Research, USA) according to Zaharia et al. (2022) protocol.

Table 3. Primers used in this study

Antibacterial compound	Gene	Primers	Primer sequence 5' - 3'	Amplification product (bp)	Annealing temperature	Reference
Iturin A	*ituA*	ITUD1 f	GATGCGATCTCCTTGGATGT	647	55°C	Sarangi et al., 2017
		ITUD1 r	ATCGTCATGTGCTGCTTGAG			
Surfactin	*srfA*	SrfA F1	AGAGCACATTGAGCGTTACAAA	626	55°C	Chung et al., 2008
		SrfA R1	CAGCATCTCGTTCAACTTTCAC			
Bacilysin	*Bac A/B*	bacA/B F	TGCTCTGTTATAGCGCGGAG	910	55°C	Compaoré et al., 2013
		bacA/B R	GTCATCGTATCCCACCCGTC			
Bacillomycin	*bmyA*	bmyA F	CTCATTGCTGCCGCTCAATC	853	55°C	Compaoré et al., 2013
		bmyA R	CCGAATCTACGAGGGGAACG			
Bacillaene	*baeA*	BaeR F	ATGTCAGCTCAGTTTCCGCA	688	55°C	Compaoré et al., 2013
		BaeR R	GATCGCCGTCTTCAATTGCC			

The PCR reaction involved one step of 4 minutes at 94°C for initial denaturation, followed by 30 cycles in three steps, one of 30 seconds at 94°C for denaturation, a second step of 30 seconds at 55°C for primers' annealing and the third step of 75 seconds at 72°C for elongation, with a final elongation step of 7 minutes at 72°C.

The amplified products (7 µl PCR product) were analyzed by 1.2 % (w/v) agarose gel electrophoresis in TBE buffer (Tris 84 mM; boric acid 89 mM; EDTA 2 mM, pH 8-8.5) containing 1 µg/ml (w/v) of ethidium bromide. The migration was performed at 100 V for 1 h, while the analysis was made under UV-light exposure, using the BioDoc-It Imaging System. Molecular weight of the bands was estimated by co-migration and band comparison with a 1 Kbp DNA ladder (ThermoScientific LSG). The PCR was scored positive when amplicons of appropriate size were detected.

RESULTS AND DISCUSSION

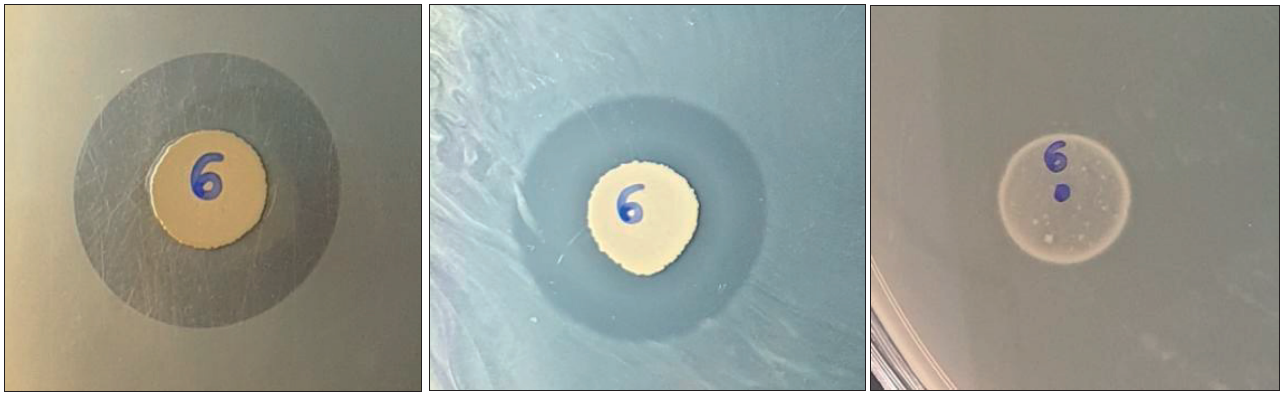
Bacterial phenotypic identification

The Biolog GEN III analysis revealed the biochemical profile of the analyzed endophytic strains. The phenotypic fingerprint of each studied bacterial strain was compared with the pattern of all references from the Biolog database, using the MicroLog3 software.

Based on their similarities, the studied strains were identified at specie level. All studied endophytic bacteria were closely related and belong to *Bacillus subtilis* group (LT MYM 1, LFF MYM 5, BPVs2, E1Pv, BAHs1 and BTAs3). The St 1T2 strain was identified as *B. pumilus*, which is also included in *Bacillus* group.

Antibacterial activity

The diffusion assay was used to determine antibacterial activity against the 24 ATCC strains, of which 19 important human and animal pathogens. When no growth developed from the pathogenic strains around the biocontrol spots, the pathogens were considered sensitive to antibacterial compounds released by the beneficial bacteria (Figure 1a). However, some pathogens could develop at reduced density in the presence of the biocontrol endophytes. In such cases pathogens were considered tolerant to the antimicrobial compounds or their dose (Figure 1b).



(a) (b) (c)

Figure 1. BAHs1 endophytic strain against different bacterial pathogens:
a) Complete inhibition of *L. monocytogenes* ATCC 7644;
b) reduced pathogenic growth of *R. equi* ATCC 6939;
c) no inhibition of *P. aeruginosa* ATCC 9027 growth.

Some of the studied strains revealed antibacterial activity against a wide spectrum of pathogens, such as: *Bacillus cereus, Enterococcus faecalis, Escherichia coli, Listeria ivanovii, L. monocytogenes, Rhodococcus equi, Salmonella enterica, S. typhimurium, Staphylococcus aureus, S. epidermidis* and *Streptococcus pyogenes* (Table 4). However, an antibacterial activity should be considered if inhibition zone has maintained for at least 1 mm in the first 48 h of co-cultivation.

Table 4. Antibacterial activity of plant beneficial endophytes used in this study

Human pathogens	Plant beneficial endophytes						
	LT MYM 1	LFF MYM 5	St 1T2	E1Pv	BPVs2	BAHs1	BTAs3
Bacillus cereus ATCC 11778	0.5 S	1.0 S	1.0 S	0.5 S+2.0 T	2.0 S	1.0 S	1.0 S
B. subtilis ATCC 6633	0	0	0	0.5 T	0.5 S+3.0 T	0.5 S	0.5 S
Ent. faecalis ATCC 29212	0	2.0 T	2.0 T	3.0 T	1.0 T	1.5 T	1.5 T
Ent. faecium ATCC 6057	1.0 T	1.0 T	0	1.5 T	1.0 T	1.0 T	2.0 T
Ent. hirae ATCC 10541	2.0 S	1.5 S	0	0	0.5 T	0.5 T	1.0 T
L. innocua ATCC 33090	2.0 T	2.0 T	1.5 T	3.0 T	2.0 S+1.0 T	2.0 T+1.0 S	3.0 T
L. ivanovii ATCC 19119	3.0 S+1.0 T	1.0 S+3.0 T	3.0 S	4.5 S	5.0 S	5.0 S	5.0 S
L. monocytogenes ATCC 7644	1.5 S	2.0 S+3.0 T	2.0 S+1.0 T	4.0 T	5.0 S	3.0 S	4.0 S
L. monocytogenes ATCC 13932	2.0 T	2.0 T	1.5 T	1.0 T	3.0 T	2.0 T	2.5 T
R. equi ATCC 6939	2.0 S	3.0 S+2.0 T	2.0 S+3.0 T	3.0 T	5.0 S	3.0 S+2.0 T	4.0 S+2.0 T
S. aureus ATCC 6538	1.5 T	1.0 T	3.5 T	0.5 T	1.0 T	2.5 T	2.5 T
S. aureus ATCC 25923	2.0 T	2.5 T	0.5 T	2.0 T	2.0 T	1.5 T	2.5 T
S. aureus ATCC 33592	2.0 T	1.5 S	3.0 T	1.5 T	1.0 T	0.5 T	1.0 T
S. aureus ATCC 43300	2.0 T	1.0 T	0.5 T	0.5 T	1.0 T	2.0 T	3.0 T
S. epidermidis ATCC 12228	2.0 T	3.0 T	3.0 T	4.0 T	2.5 T	3.0 T	4.0 T
S. epidermidis ATCC 51625	1.5 T	3.0 T	5.0 T	2.0 T	3.0 T	3.0 T	4.0 T
Streptococcus pyogenes ATCC 19615	0	1.0 T	3.0 T	0	3.0 T	2.0 T	4.0 T
Citrobacter freundii ATCC 43864	0.5 T	1.0 T	0	0	1.0 T	1.0 T	0.5 T
Escherichia coli ATCC 8739	1.0 T	1.0 T	0	0.5 T	1.0	1.0 T	0.5 T
E. coli F. ATCC 25922	2.0 T	2.0 T	2.0 T	0	2.0 S	1.0 T	2.0 T
Proteus hauseri ATCC 13315	2.5 T	2.0 T	0	1.5 T	1.5 T	2.0 T	2.0 T
Pseudomonas aeruginosa ATCC 9027	0	0	0	0	0	0	0
Sal. enterica ATCC 13076	2.0 T	3.0 T	1.5 T	4.0 T	2.0 T	2.0 T	3.0 T
Sal. typhimurium ATCC 14028	1.0 T	1.0 T	0	1.0 T	1.0 T	1.0 T	1.0 T

Legend: S = sensitive, no pathogenic growth was developed; T = tolerant, the pathogenic growth was reduced; 0 - resistance, the pathogenic growth was not disturbed.

The endophytic, plant beneficial strains were more efficient in suppressing Gram positive pathogenic bacteria. Against *B. cereus, L. ivanovii, L. monocytogenes* and *R. equi* the effect was bactericidal. The pathogenic growth was completely suppressed for up to 5 mm radius around the endophyte spots, depending on the tested strains. In all other cases of antibacterial activity, the pathogens were not completely suppressed, but only delayed, as they could developed at reduced density. Among the tested strains, best results were obtained with the lavender and seeds endophytes.

Molecular detection of antibacterial functional genes

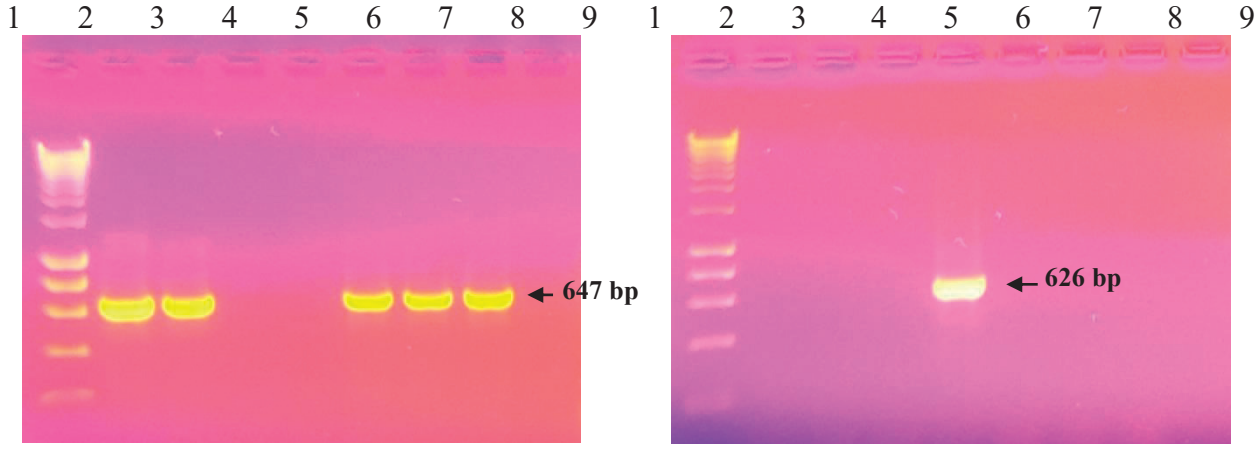
The endophytic bacterial strains were analyzed through molecular techniques in order to detect five functional genes (Table 5), each encoding for a different antimicrobial compound (iturin A, surfactin, bacilysin, bacillomycin and bacillaene).

Table 5. Molecular detection of the genes encoding antimicrobial compounds

Endophytic strain	Functional gene				
	ituA	*srfA*	*bacA/B*	*bmyA*	*baeA*
LT MYM 1	+	–	+	+	+
LFF MYM 5	+	–	+	+	+
St 1T2	–	–	–	–	–
E1Pv	–	+	–	–	+
BPVs2	+	–	+	+	+
BAHs1	+	–	+	+	+
BTAs3	+	–	+	+	+

Legend: present (+) / absent (–) functional gene.

Regarding endophytes potential to produce antimicrobial compounds, best results were obtained with those strains isolated from the lavender and seeds (LT MYM 1, LFF MYM 5, BPVs2, BAHs1 and BTAs3). They are able to produce iturin A (Figure 2a), surfactin, bacilysin, bacillomycin and bacillaene. These results explaining the higher antibacterial activity of this strains. The gene encoding for surfactin synthesis was detected only in E1Pv strain (Figure 2b).



(a) (b)

Figure 2. Example of electrophoretic profiles of the PCR products revealing *ituA* (a) and *srfA* (b) genes
Lines: 1 – 1 Kbp DNA marker, 2 – LT MYM 1 strain, 3 – LFF MYM 1 strain, 4 – St 1T2 strain, 5 – E1Pv strain, 6 – BPVs2 strain, 7 – BAHs1 strain, 8 – BTAs3 strain, 9 – Negative control.

In the present study, the seven endophytic bacteria were identified based on the phenotypic/biochemical profiles obtained in the Biolog GEN III MicroPlate assay. Thus, it was revealed they belong to the *Bacillus subtilis* group and *B. pumilus*. Identifying endophytic bacteria from *Bacillus subtilis* group is a common aspect revealed in many studies (Xia et al., 2013; Lopes et al., 2015; Jasim et al., 2016; Bolivar-Anillo et al., 2021), some of them highly reliable, as their genome was complete or partial sequenced (Deng et al., 2011; Jeong et al., 2014; Sun et al., 2015; Cai et al., 2016). *Bacillus* spp. are ubiquitous bacteria; they could be found in various environments (Schultz et al., 2017), including as endophytes (Gond et al., 2015; Shahzad et al., 2016; Boiu-Sicuia and Cornea, 2019; Cheng et al., 2020).

Although *Bacillus* spp. are known as promising biocontrol microorganisms (Etesami and Alikhani, 2018). Several studied mention them also to have the ability to inhibit human and animal pathogens, such as *Bacillus cereus*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *S. typhimurium* and *Staphylococcus aureus* (Avcı et al., 2016). However, the *Bacillus* strains used in this study were not able to inhibit *Pseudomonas aeruginosa* growth. Our strains were more efficient against Gram-positive pathogenic bacteria, some strains having bactericidal activity against *B. cereus*, *L. ivanovii*, *L. monocytogenes*, and *Rhodococcus equi*.

Most studies revealing beneficial bacteria inhibiting or suppressing human and animal pathogens are those focused in lactic acid bacteria (Vătuiu and Popa, 2015). Although these bacteria are generally recognized as safe (de Lacerda et al., 2016), their use as agro-inoculants is limited. Still, *Bacillus* are having multiple qualities, they are having wide ecological plasticity, are spore forming bacteria (Kefi et al., 2015), and can produce a wide variety of enzymes (Su et al., 2020) and biologic active compounds (Ek-Ramos et al., 2019).

An important aspect for which they are used as biocontrol agents is their ability to produce various antimicrobial compounds with antifungal and antibacterial activity. Among the important metabolites of *Bacillus* spp. that demonstrated antibacterial activity are bacteriocines and certain classes of lipopeptides, such as iturins and surfactins, as well as bacilysin and bacitracin A and F (Caulier et al., 2019).

In this study, the lavender and seeds endophytes (LT MYM 1, LFF MYM 5, BPVs2, BAHs1 and BTAs3) revealed to possess genes involved in iturin A, bacilysin, bacillomycin and bacillaene antimicrobial compounds synthesis. Such compounds are mentioned as antibacterial compounds in various studies (Fira et al., 2018; Patel et al., 1995), thus sustaining the results.

CONCLUSIONS

Selected endophytic *Bacillus* strains could be considered promising biocontrol agents due to their antimicrobial activity. The selected lavender and seeds endophytes (LT MYM 1, LFF MYM 5, BPVs2, BAHs1 and BTAs3) could be used in both conventional and organic farming as they are generally recognized as safe microbial inoculants. They revealed antagonistic activity against important multidrug resistant and highly virulent human and animal pathogens. Moreover, their antibiotic compounds are of different nature than those of pharmaceutical use, thus they have no restrictions to be used in agriculture.

ACKNOWLEDGEMENTS

This research was funded by the University of Agronomic Sciences and Veterinary Medicine of Bucharest, project number 2021-0005/13.07.2021 - Developing a microbial bio-fertilizer technology.

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