Original Research Article: full paper

(2021), «EUREKA: Life Sciences»
Number 4

SURVEY OF PLANT GROWTH PROMOTING AND ANTAGONISTIC TRAITS IN WINTER WHEAT GRAIN ENDOPHYTIC BACTERIA

Alina Pastoshchuk[1]✉
kotsyuk93@ukr.net

Yuliia Yumyna[1]

Pavlyna Zelena[1]

Larysa Skivka[1]

[1]Department of Microbiology and Immunology
Educational and Scientific Centre "Institute of Biology and Medicine"
Taras Shevchenko National University of Kyiv
64/13 Volodymyrska str., Kyiv, Ukraine, 01601

✉**Corresponding author**

Abstract

The aim of this work was to isolate endophytic bacteria from wheat grains and to evaluate their plant growth promoting traits (PGPT) as well as an inhibitory effect on *P. syringae pv. atrofaciens* (McCulloch) growth. Endophytic bacteria were isolated by a culture-dependent protocol from the grains of winter wheat variety of Ukrainian selection Podolyanka with high resistance to syringae. Totally 2.7±0.09 CFU/1 g of dry wheat grain were isolated, ten cultivable bacterial isolates were obtained. Spore-forming bacilli predominated in the wheat grain endophytic community. Gram-negative fermenting and non-fermenting rod-shaped bacteria and Gram-positive cocci were also present. Seven out of ten isolates possessed numerous plant growth promoting traits including phosphate solubilization, oligonitrotrophy, and indolic compound producing. Two isolates possessed antagoniscic activity against syringae *in vitro* along with plant growth promoting features. According to biochemical profiling and mass-spectrophotometric identification, these two isolates were assigned to *Paenibacillus* and *Brevibacillus* genera. These endophytic bacteria can be considered as promising objects for agrobiotechnology. However, more research is needed to confirm their biotechnological potential *in planta* experiments.

Keywords: wheat grain endophytic bacteria, plant growth promoting traits, antagonistic activity, *Pseudomonas syringae pv. atrofaciens* (McCulloch).

DOI: 10.21303/2504-5695.2021.001978

1. Introduction

It is generally accepted, that bacterial endophytic communities are present in all plants [1, 2]. The growing number of studies on endophytic bacteria has revealed their significance in agricultural production, food safety, and phytoremediation. Namely, plant endophytic microbiome is associated with improved plant productivity. Endophytic bacteria get nutrients from soils and transfer them to host tissues in different nutrient-transfer symbioses, increase plant growth and development producing and/or up-regulating growth hormones etc. In addition, plant endophytic bacteria protect host from infectious diseases and herbivores, and even deter growth of competitor plant species [3, 4]. Endophytic bacterial communities of some root vegetables contain lactic acid bacteria, offering its use as a source of probiotic microorganisms [5]. Endophytes are involved in health-beneficial effects of phytoremedies from medicinal plants [6]. Some endophytic bacteria exhibit organic pollutant degradation activity along with plant growth-promoting properties, and can be successfully applied for intensified phytoremediation [7]. All these features of endophytic bacteria position them as a promising object for biotechnology.

Currently, the overwhelming majority of bacterial endophytes is non-cultivable (only 0.001–1 % of all plant-associated endophytic bacteria are cultivable), and therefore are not appropriate for biotechnological employment [8]. Meanwhile, cultivable endophytic bacteria are extensively investigated in the prospect of their use in the agriculture system, as well as in medicine and nanobiotechnology [9, 10]. Considering close interrelation between endophytic bacteria and their host plants, endophytes could potentially be used as biological control agents in sustainable crop production [11], including cereal protection [12]. Biocontrol agents, based on endophytic bacteria, can stimulate cereal growth and inhibit disease development through various mechanisms, including plant immunity regulation and direct antagonistic activity against phytopathogens [13].

Wheat (*Triticum aestivum L.*) is a strategic grain crop for Ukraine. Ukraine is currently among ten largest wheat producing countries in the world [14]. Growing global demand for cereals for food and feed, and as a source for biofuels necessitates obtaining high yields of this grain crop. More than 50 diseases reduce wheat grain yield by approximately 18–20 % [15]. Among others, numerous bacterial wheat diseases (bacterial spot, speck, and blight) are caused by *Pseudomonas syringae*, namely *P. syringae pv. atrofaciens* (McCulloch) – phytopathogenic bacterium with high-grade resistance to antimicrobial compounds [16]. It foregrounds the search of alternative agents for biocontrol of this phytopathogen, such as biopreparations based on endophytic bacteria.

The aim of this work was to isolate endophytic bacteria from wheat grains and to evaluate their plant growth promoting traits (PGPT) as well as an inhibitory effect on *P. syringae pv. atrofaciens* (McCulloch) growth.

2. Materials and Methods

2. 1. Isolation, morphological, physiological and biochemical characters, and identification of cultivable endophytic bacteria from wheat grain

Wheat grains were first rinsed with running tap water for 15 min, then were surface asepticized with 72 % ethanol for 30 s, followed by treatment with 2 % trichloroisocyanuric acid for 4,5 min and finally – with 72 % ethanol for 30 s. After this, grains were rinsed six times for 15 min with sterilized distilled water by shaking at 220 rpm. 1 mL of sterile water, used for the final washing, was then plated onto R2A agar and incubated for 7 days at 28 °C in order to test grain surface sterilization efficacy and epiphytic bacteria removal. After this, 1 g of sterilized wheat grains was homogenized in 10 mM phosphate buffer (pH 6.5). A serial dilution of homogetane was made and plated on potato agar for total bacteria count, on McConkie agar (HiMedia) for Gram-negative bacteria count, and on MYP agar (HiMedia) for bacilli count. Plates were cultured at 24 °C. For the isolate characterization 10 sterilized grains were put on Petri dishes with R2A and cultured for 72 h at 28 °C (**Fig. 1**).

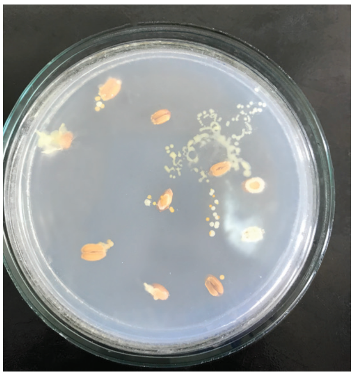


Fig. 1. Image illustrates representative morphotypes of endophyte colonies, isolated from wheat grain, variety Podolyanka

Representative colonies were characterized according to their morphological features, including shape, margin, elevation, viscosity, color, opacity etc. Obtained bacterial isolates were then tested for potential PGPT. The phosphate solubilizing activity was examined using Muromtsev agar [17]. The oligonitrotrophic activity was detected using nitrogen-free Ashby's mannitol agar [18]. The capability to produce indolic compounds was examined by isolate culturing in the Stroganov liquid medium, supplemented with L-tryptophan, Salkowski's reagent was used to reveal indolic compound [19]. MALDI-TOF mass spectrometry and biochemical profiling were used for the identification of isolated microorganisms. MALDI-TOF mass spectrometry was performed using VITEK MS (bioMérieux SA, France) according to the manufacturer recommendations. Spectra were generated using the MYLA software version 3.0.0, MYLA version 4.6.1. The range of probabilities for correct identification was 60 to 99 % with values closer to 99.9 %. The confidence level was determined with percent probability and number of choices [20]. Biochemical profiling was conducted using VITEK® 2Compact (bioMérieux SA, France) according to the system manufacturer recommendations.

2. 2. Antagonistic activity assessment

The antagonistic activity of isolated endophytic bacteria against *P. syringae pv. atrofaciens* (McCulloch) was tested *in vitro* using agar well diffusion assay [21]. A strain of *P. syringae pv. atrofaciens* (McCulloch) Young, Dye & Wilkie 1978: UKM B-1013 was grown on potato agar at 28 °C for 24–48 hours. The overnight culture was used for the experiment.

2. 3. Statistical analysis

All experimental data are presented as mean ± SD of at least three independent experiments. Statistical significance of the differences between two groups was determined by Student's t-test. Differences were considered significant at $p \leq 0.05$.

3. Results and Discussion

In previous experiments we have revealed winter wheat variety of Ukrainian selection Podolianka with high resistance to *P. syringae pv. atrofaciens* (McCulloch) [22]. Grain invasion in wheat agrophytocenosis is an important component in the epidemiology of bacterioses, caused by *P. syringae pv. atrofaciens* (McCulloch). Endophytic microorganisms of grain are considered as one of the most important resistance-inducing determinants of the plant resistance to the phytopathogen [23]. Considering this, grains of Podolyanka wheat variety were chosen for the search of endophytic bacteria with PGPT and antagonistic activity against phytopathogenic bacteria. Totally 2.7±0.09 CFU/1 g of dry wheat grain were isolated, ten cultivable bacterial isolates (P1–10) were obtained. Morphotypes of isolated grain endophytic bacteria were represented by endospore-forming bacilli (5 isolates), gram-negative fermenting and non-fermenting rods (2 and 1 isolates respectively), and gram-positive cocci (2 isolates). Different PGPT were revealed in seven out of ten isolates (**Table 1**).

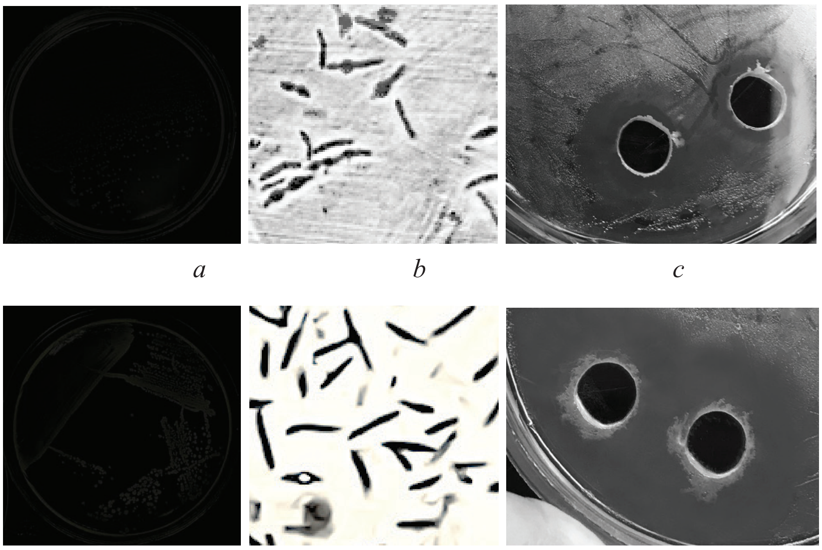
Table 1

Plant growth promoting traits of endophytic bacteria

Isolate No	Morphotype	Gram staining	Phosphate solubilization	Oligonitrotrophy	Indolic compounds
1	2	3	4	5	6
P1	spore-forming bacilli	positive	+	+	–
P2	spore-forming bacilli	positive	–	–	+
P3	cocci	positive	–	-	-
P4	rods	negative	+	+	+
P5	rods	negative	+	–	+
P6	spore-forming bacilli	positive	+	+	–
P7	cocci	positive	–	–	–
P8	spore-forming bacilli	positive	–	–	–
P9	rods	negative	+	+	+
P10	spore-forming bacilli	positive	–	–	+

Three isolates (P3 and P7 cocci, and spore-forming bacillus P8) showed no PGPT. Two spore-forming bacilli (P2 and P10) were capable to produce indolic compounds. Spore-forming bacilli P1 and P6 are phosphate-solubilizing oligonitrotrophes. P5 is phosphate-solubilizing Gram-negative rod with the ability to produce indolic compounds. P4 and P6 are Gram-negative rods, possessing three PGPT: phosphate-solubilizing activity, oligonitrotrophy and potent capability to produce indolic compounds.

The antagonistic activity against *P. syringae pv. atrofaciens* (McCulloch) was found out in two grain endophytic bacterial isolates (P6 and P10) by observing characteristic clear zones, inhibiting the growth of the pathogen around the well (**Fig. 2**). P10 exhibited the maximum inhibition (inhibition zone 2.7±0.3 mm), P6 – 2.3±0.1 mm.



a *b* *c*

d *e* *f*

Fig. 2. Image illustrates: *a*, *d* – colony morphotype; *b*, *e* – cell morphology; *c*, *f* – antagonistic activity of endophytic bacterial isolates P6 (*a*, *b*, *c*) and P10 (*d*, *e*, *f*)

Endophytic bacteria, possessing PGPT along with antagonistic activity against phytopathogenic bacterium, can be considered as more forceful allies and more efficient mates of host plant, as well as seem to be more promising objects for agricultural biotechnology [24, 25]. For these reasons, we have conducted biochemical and mass-spectrophotometric identification of P6 and P10 isolates. According to the biochemical profiling, endophytes with antagonistic activity belonged to *Paenibacillus* and *Brevibacillus* genera (**Table 2**).

The belonging of the isolates to the specified genera is confirmed by the morphology of the cells: terminal to sub-terminal endospore location in *Paenibacillus peoriae* and central – in *Brevibacillus brevis* (**Fig. 2, *b*, *e***). In addition, the results of biochemical identification were confirmed by the mass-spectrophotometric assays (**Table 3**). Our findings are concordant with literature data concerning the antagonistic activity of bacteria, belonging to *Paenibacillus* and *Brevibacillus* genera against phytopathogens. *Brevibacillus brevis* exerts the antagonistic effect towards *Phytophthora nicotianae* [26] and *Fusarium* spp. [27]. *Paenibacillus* are characterized by the potent antagonistic activity against soil-borne phytopathogens [28] and *Fusarium* [29].

This study has potential limitations. The examination of antagonistic activity of isolated wheat grain endophytic bacteria was performed only in *in vitro* experiments, and warrants validation in *in planta* condition. Moreover, one can't exclude that endophytic bacteria exert more powerful PGP and antagonistic activity in the consortium than used alone. In addition, biochemical and mass-spectrophotometric isolate identification should be confirmed using 16S rRNA Gene Sequencing.

Table 2

Biochemical profiles of endophytic bacterial isolates using VITEK/BCL card

Isolate P6. *Paenibacillus peoriae.* Sample ID 5352550515047161. Confidence value 89 %. Confidence level: good identification																	
1	BXYL	+	3	LysA	–	4	AspA	+	5	LeuA	+	7	PheA	+	8	ProA	–
9	BGAL	+	10	PyrA	–	11	AGAL	+	12	AlaA	–	13	TyrA	(–)	14	BNAG	–
15	APPA	+	18	CDEX	–	19	dGAL	+	21	GLYG	+	22	INO	–	24	MdG	+
25	ELLM	–	26	MdX	–	27	AMAN	–	29	MTE	+	30	GlyA	–	31	dMAN	–
32	dMNE	+	34	dMLZ	–	36	NAG	–	37	PLE	+	39	lRHA	–	41	BGLU	+
43	BMAN	–	44	PHC	–	45	PVATE	–	46	AGLU	–	47	dTAG	–	48	dTRE	+
50	INU	+	53	dGLU	+	54	dRIB	+	56	PSCNa	–	58	NaCl 6.5 %	–	59	KAN	+
60	OLD	+	61	ESC	+	62	TTZ	(+)	63	POLYB_R	+	–	–	–	–	–	–
Isolate P10. *Brevibacillus brevis/ Brevibacillus agri.* Sample ID 4420100000200401. Confidence value 87 %. Confidence level: acceptable identification																	
1	BXYL	–	3	LysA	–	4	AspA	(+)	5	LeuA	–	7	PheA	(–)	8	ProA	+
9	BGAL	–	10	PyrA	+	11	AGAL	–	12	AlaA	–	13	TyrA	–	14	BNAG	–
15	APPA	+	18	CDEX	–	19	dGAL	–	21	GLYG	–	22	INO	–	24	MdG	–
25	ELLM	–	26	MdX	–	27	AMAN	–	29	MTE	–	30	GlyA	–	31	dMAN	(–)
32	dMNE	–	34	dMLZ	–	36	NAG	–	37	PLE	–	39	lRHA	–	41	BGLU	–
43	BMAN	–	44	PHC	+	45	PVATE	–	46	AGLU	–	47	dTAG	–	48	dTRE	–
50	INU	–	53	dGLU	–	54	dRIB	–	56	PSCNa	–	58	NaCl 6.5 %	–	59	KAN	+
60	OLD	–	61	ESC	–	62	TTZ	–	63	POLYB_R	+	–	–	–	–	–	–

Table 3

Identification of endophytic bacteria from wheat grains by the MALDI-TOF mass-spectrometry

Isolate No	P6	P10
Organism name	*Paenibacillus peoriae*	*Brevibacillus spp.*
Slide ID	202012041	202012041
Confidence value, %	99.9	99.9
Confidence level	High	High

4. Conclusions

1. Our data confirm the presence of rich bacterial endophyte in winter wheat grains, in which spore-forming bacilli predominate.

2. Some cultivable endophytic bacteria possess two and even three PGPT simultaneously.

3. In this preliminary study, we have revealed two bacterial isolates with PGPT and antagonistic activity towards *P. syringae pv. atrofaciens* (McCulloch), which can be considered as promising agents for agrobiotechnology.

Acknowledgement

We would like to certify our special acknowledgement to Valentyna Yanovska and Valentyn Nudha for the help in the mass-spectrophotometric assays.

Conflicts of Interest

Authors declare no conflict of interest.

References

[1] Liu, H., Carvalhais, L. C., Crawford, M., Singh, E., Dennis, P. G., Pieterse, C. M. J., Schenk, P. M. (2017). Inner Plant Values: Diversity, Colonization and Benefits from Endophytic Bacteria. Frontiers in Microbiology, 8. doi: https://doi.org/10.3389/fmicb.2017.02552

[2] Oukala, N., Aissat, K., Pastor, V. (2021). Bacterial Endophytes: The Hidden Actor in Plant Immune Responses against Biotic Stress. Plants, 10 (5), 1012. doi: https://doi.org/10.3390/plants10051012

[3] White, J. F., Kingsley, K. L., Zhang, Q., Verma, R., Obi, N., Dvinskikh, S. et. al. (2019). Review: Endophytic microbes and their potential applications in crop management. Pest Management Science, 75 (10), 2558–2565. doi: https://doi.org/10.1002/ps.5527

[4] Singh, D. P., Gupta, V. K., Prabha, R. (Eds.) (2019). Microbial Interventions in Agriculture and Environment. Springer, 573. doi: https://doi.org/10.1007/978-981-13-8383-0

[5] Kõiv, V., Arbo, K., Maiväli, Ü., Kisand, V., Roosaare, M., Remm, M., Tenson, T. (2019). Endophytic bacterial communities in peels and pulp of five root vegetables. PLOS ONE, 14 (1), e0210542. doi: https://doi.org/10.1371/journal.pone.0210542

[6] Erjaee, Z., Shekarforoush, S. S., Hosseinzadeh, S. (2019). Identification of endophytic bacteria in medicinal plants and their antifungal activities against food spoilage fungi. Journal of Food Science and Technology, 56 (12), 5262–5270. doi: https://doi.org/10.1007/s13197-019-03995-0

[7] Singh, T., Awasthi, G., Tiwari, Y. (2021). Recruiting endophytic bacteria of wetland plants to phytoremediate organic pollutants. International Journal of Environmental Science and Technology. doi: https://doi.org/10.1007/s13762-021-03476-y

[8] Eevers, N., Gielen, M., Sánchez-López, A., Jaspers, S., White, J. C., Vangronsveld, J., Weyens, N. (2015). Optimization of isolation and cultivation of bacterial endophytes through addition of plant extract to nutrient media. Microbial Biotechnology, 8 (4), 707–715. doi: https://doi.org/10.1111/1751-7915.12291

[9] Rana, K. L., Kour, D., Kaur, T., Devi, R., Yadav, A. N., Yadav, N. et. al. (2020). Endophytic microbes: biodiversity, plant growth-promoting mechanisms and potential applications for agricultural sustainability. Antonie van Leeuwenhoek, 113 (8), 1075–1107. doi: https://doi.org/10.1007/s10482-020-01429-y

[10] Rana, K. L., Kour, D., Yadav, N., Yadav, A. N. (2020). Endophytic microbes in nanotechnology: Current development, and potential biotechnology applications. Microbial Endophytes, 231–262. doi: https://doi.org/10.1016/b978-0-12-818734-0.00010-3

[11] Tao, A., Pang, F., Huang, S., Yu, G., Li, B., Wang, T. (2014) Characterisation of endophytic Bacillus thuringiensis strains isolated from wheat plants as biocontrol agents against wheat flag smut. Biocontrol Science and Technology, 24 (8), 901–924. doi: https://doi.org/10.1080/09583157.2014.904502

[12] Pan, D., Mionetto, A., Tiscornia, S., Bettucci, L. (2015). Endophytic bacteria from wheat grain as biocontrol agents of Fusarium graminearum and deoxynivalenol production in wheat. Mycotoxin Research, 31 (3), 137–143. doi: https://doi.org/10.1007/s12550-015-0224-8

[13] Newitt, J., Prudence, S., Hutchings, M., Worsley, S. (2019). Biocontrol of Cereal Crop Diseases Using Streptomycetes. Pathogens, 8 (2), 78. doi: https://doi.org/10.3390/pathogens8020078

[14] Ryabchenko, O., Nonhebel, S. (2016). Assessing wheat production futures in the Ukraine. Outlook on Agriculture, 45 (3), 165–172. doi: https://doi.org/10.1177/0030727016664159

[15] Singh, R. P., Singh, P. K., Rutkoski, J., Hodson, D. P., He, X., Jørgensen, L. N. et. al. (2016). Disease Impact on Wheat Yield Potential and Prospects of Genetic Control. Annual Review of Phytopathology, 54 (1), 303–322. doi: https://doi.org/10.1146/annurev-phyto-080615-095835

[16] Valencia-Botín, A. J., Cisneros-López, M. E. (2012). A Review of the Studies and Interactions of Pseudomonas syringae Pathovars on Wheat. International Journal of Agronomy, 201, 1–5. doi: https://doi.org/10.1155/2012/692350

[17] Alikhani, H. A., Saleh-Rastin, N., Antoun, H. (2006). Phosphate solubilization activity of rhizobia native to Iranian soils. Plant and Soil, 287 (1-2), 35–41. doi: https://doi.org/10.1007/s11104-006-9059-6

[18] Woźniak, M., Gałązka, A., Tyśkiewicz, R., Jaroszuk-Ściseł, J. (2019). Endophytic Bacteria Potentially Promote Plant Growth by Synthesizing Different Metabolites and their Phenotypic/Physiological Profiles in the Biolog GEN III MicroPlateTM Test. International Journal of Molecular Sciences, 20 (21), 5283. doi: https://doi.org/10.3390/ijms20215283

[19] Abdelaziz, S., Hemeda, N. F., Belal, E. E., Elshahawy, R. (2018). Efficacy of Facultative Oligotrophic Bacterial Strains as Plant Growth-Promoting Rhizobacteria (PGPR) and their Potency Against Two Pathogenic Fungi Causing Damping-off Diseases. Applied Microbiology: Open Access, 04 (03). doi: https://doi.org/10.4172/2471-9315.1000153

[20] Rychert, J., Burnham, C.-A. D., Bythrow, M., Garner, O. B., Ginocchio, C. C., Jennemann, R. et. al. (2013). Multicenter Evaluation of the Vitek MS Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry System for Identification of Gram-Positive Aerobic Bacteria. Journal of Clinical Microbiology, 51 (7), 2225–2231. doi: https://doi.org/10.1128/jcm.00682-13

[21] Agarwal, H., Dowarah, B., Baruah, P. M., Bordoloi, K. S., Krishnatreya, D. B., Agarwala, N. (2020). Endophytes from Gnetum gnemon L. can protect seedlings against the infection of phytopathogenic bacterium Ralstonia solanacearum as well as promote plant growth in tomato. Microbiological Research, 238, 126503. doi: https://doi.org/10.1016/j.micres.2020.126503

[22] Pastoshchuk, A. Yu., Skivka, L. M., Butsenko, L. M., Patyka, V. P. (2018). Effect of causal agent of basal bacteriosis on seed germination and root growth of different wheat varieties. Microbiology&Biotechnology, 2 (42), 39–48. doi: https://doi.org/10.18524/2307-4663.2018.2(42).134449

[23] Xing, Z., Wu, X., Zhao, J., Zhao, X., Zhu, X., Wang, Y. et. al. (2020). Isolation and identification of induced systemic resistance determinants from Bacillus simplex Sneb545 against Heterodera glycines. Scientific Reports, 10 (1). doi: https://doi.org/10.1038/s41598-020-68548-4

[24] El-Sayed, W. S., Akhkha, A., El-Naggar, M. Y., Elbadry, M. (2014). In vitro antagonistic activity, plant growth promoting traits and phylogenetic affiliation of rhizobacteria associated with wild plants grown in arid soil. Frontiers in Microbiology, 5. doi: https://doi.org/10.3389/fmicb.2014.00651

[25] Karthika, S., Varghese, S., Jisha, M. S. (2020). Exploring the efficacy of antagonistic rhizobacteria as native biocontrol agents against tomato plant diseases. 3 Biotech, 10 (7). doi: https://doi.org/10.1007/s13205-020-02306-1

[26] Jin, F., Ding, Y., Ding, W., Reddy, M. S., Fernando, W. G. D., Du, B. (2011). Genetic Diversity and Phylogeny of Antagonistic Bacteria against Phytophthora nicotianae Isolated from Tobacco Rhizosphere. International Journal of Molecular Sciences, 12 (5), 3055–3071. doi: https://doi.org/10.3390/ijms12053055

[27] Alizadeh, M., Vasebi, Y., Safaie, N. (2020). Microbial antagonists against plant pathogens in Iran: A review. Open Agriculture, 5 (1), 404–440. doi: https://doi.org/10.1515/opag-2020-0031

[28] Wang, X., Li, Q., Sui, J., Zhang, J., Liu, Z., Du, J. et. al. (2019). Isolation and Characterization of Antagonistic Bacteria Paenibacillus jamilae HS-26 and Their Effects on Plant Growth. BioMed Research International, 2019, 1–13. doi: https://doi.org/10.1155/2019/3638926

[29] Zhai, Y., Zhu, J., Tan, T., Xu, J., Shen, A., Yang, X. et. al. (2021). Isolation and characterization of antagonistic Paenibacillus polymyxa HX-140 and its biocontrol potential against Fusarium wilt of cucumber seedlings. BMC Microbiology, 21 (1). doi: https://doi.org/10.1186/s12866-021-02131-3

Received date 21.05.2021
Accepted date 09.07.2021
Published date 30.07.2021

How to cite: *Pastoshchuk, A., Yumyna, Y., Zelena, P., Skivka, L. (2021). Survey of plant growth promoting and antagonistic traits in winter wheat grain endophytic bacteria. EUREKA: Life Sciences, 4, 66–72. doi: https://doi.org/10.21303/2504-5695.2021.001978*