

## PLANT GROWTH PROMOTING BACTERIAL ENDOPHYTES ISOLATED FROM POLISH HERBAL PLANTS

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### ABSTRACT

Endophytes produce a wide range of compounds with high application potential, mainly in medicine and agriculture. In this study, we test the hypothesis that endophytic bacteria produce indole-3-acetic acid (IAA), have positive influence on plant root development and are possible to application as plant-growth promoters. Endophytic bacteria were isolated from 3 native growing plant species: *Chelidonium majus* L., *Elymus repens* L., *Solidago gigantea* L. All endophytic strains produced IAA and the highest levels of IAA were observed for *Pseudomonas azotoformans* P3 strain. Triticale seed bacterization did not affect the seed germination, but had significant influence on root length and the longest roots were obtained after seed treatment with *Pseudomonas* sp. strains. Triticale roots were longer only in seedlings grown from seeds treated with endophytic strains producing high IAA levels (more than 22  $\mu\text{g ml}^{-1}$ ). Our results suggest that endophytic *Pseudomonas* sp. strains isolated from *Elymus repens* L. can be used as plant-growth promoter.

**Key words:** endophytes, growth promotion, herbal plants, *Chelidonium majus* L., *Elymus repens* L., *Solidago gigantea* L.

### INTRODUCTION

Endophytic microorganisms are plant-associated bacteria or fungi that colonize internal tissues of plants without causing any harm to them. They have been isolated from above-ground parts of plants, like stems, flowers, leaves and fruits. According to many authors, microorganisms isolated from roots and seeds are also endophytes [Reinhold-Hurek and Hurek 1998, Tan and Zou 2001]. Nowadays, as deforestation and biodiversity loss is widespread, thousands of endophytes will be lost before being explored.

Since the early 90s of the twentieth century, screening for endophytes intensified very much. En-

dophytes were isolated for the first time more than 100 years ago. In 1904, Freeman obtained endophytic fungus from seeds of annual ryegrass (*Lolium temulentum* L.). Two years later, fungi belonging to the genus *Neotyphodium* were isolated from green parts of the plant. Recent studies have shown that these fungi produce insecticidal alkaloids [Schardl et al. 2007]. In the early twentieth century, the knowledge about endophytes and their interactions with host plant has been much developed, but we still do not understand everything. With respect to phylogenetic affiliation of bacterial endophytes, they mainly be-

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**Table 1.** Phylogenetic affiliation of endophytic bacteria [Zinniel et al. 2002, Hung and Annapurna 2004, Rosenblueth and Martínez-Romero 2006, Romero et al. 2014, Shi et al. 2014]

Phylum/class	Genus
$\alpha$ -Proteobacteria	<i>Acetobacter</i> , <i>Agrobacterium</i> , <i>Azorhizobium</i> , <i>Azospirillum</i> , <i>Bradyrhizobium</i> , <i>Gluconacetobacter</i> , <i>Methylobacterium</i> , <i>Rhizobium</i> , <i>Sinorhizobium</i> , <i>Sphingomonas</i>
$\beta$ -Proteobacteria	<i>Alcaligenes</i> , <i>Azoarcus</i> , <i>Burkholderia</i> , <i>Chromobacterium</i> , <i>Herbaspirillum</i>
$\gamma$ -Proteobacteria	<i>Aeromonas</i> , <i>Citrobacter</i> , <i>Enterobacter</i> , <i>Erwinia</i> , <i>Escherichia</i> , <i>Klebsiella</i> , <i>Pantoea</i> , <i>Pseudomonas</i> , <i>Salmonella</i> , <i>Serratia</i> , <i>Stenotrophomonas</i> , <i>Xanthomonas</i>
Firmicutes	<i>Bacillus</i> , <i>Clostridium</i> , <i>Leuconostoc</i> , <i>Paenibacillus</i> , <i>Staphylococcus</i>
Bacteroidetes	<i>Sphingobacterium</i>
Actinobacteria	<i>Arthrobacter</i> , <i>Cellulomonas</i> , <i>Clavibacter</i> , <i>Corynebacterium</i> , <i>Curtobacterium</i> , <i>Kocuria</i> , <i>Microbacterium</i> , <i>Micrococcus</i> , <i>Mycobacterium</i> , <i>Nocardia</i> , <i>Rothia</i> , <i>Streptomyces</i> , <i>Tsukamurella</i>

long to the following types: *Proteobacteria* ( $\alpha$ -,  $\beta$ -,  $\gamma$ -,  $\delta$ -,  $\epsilon$ -*Proteobacteria*), *Firmicutes*, *Actinobacteria*, *Acidobacteria* and *Bacteroidetes* (Tab. 1). The most common are endophytes classified to *Proteobacteria*, while the most commonly found genera of endophytes are *Pseudomonas*, *Bacillus*, *Burkholderia*, *Stenotrophomonas*, *Micrococcus*, *Pantoea* and *Microbacterium* [Romero et al. 2014, Shi et al. 2014, Santoyo et al. 2016].

To date, scientists are working on endophytic genomes sequencing as only few complete genomes have been published. Sequenced endophytic genomes include *Azoarcus* sp. BH72, *Azospirillum lipoferum* 4B, *Burkholderia phytofirmans* PsJN, *Burkholderia* spp. KJ006, *Enterobacter cloacae* ENHKU01, *Enterobacter* sp. 638, *Gluconobacter diazotrophicus* Pa15, *Klebsiella pneumoniae* 342, *Pseudomonas putida* W619, *Pseudomonas stutzeri* A1501, *Serratia proteamaculans* 56 and *Stenotrophomonas maltophilia* R551-3. [Krause et al. 2006, Fouts et al. 2008, Yan et al. 2008, Bertalan et al. 2009, Taghavi et al. 2009, Weilharter et al. 2011, Wiśniewski et al. 2011, Kwak et al. 2012, Liu et al. 2012, ]. Sequences of endophytic genomes can be compared with those of bacteria belonging to similar species while inhabiting different environments. This method enable to discover genes involved in specific interaction between endophytes and a host plant. Using bioinformatic methods, scientists found more than 40 putative genes involved in endophytic behavior, which included

transporter proteins encoding genes, secretion and delivery systems, plant polymer degradation or modification etc. [Ali et al. 2014].

Many investigations proved that endophytes stimulate plant growth. In the presence of endophytes, plants are protected from pathogens attack and grow faster. Furthermore, plants inhabiting endophytes are better adapted to adverse environmental conditions such as drought or presence of heavy metals in the environment. Some endophytes enhance the effect of allelopathy in plants and therefore the dominance of plants promoted by endophytes is a common phenomenon in many environments [Tan and Zou 2001]. Plant growth-promoting endophytes (PGPE) facilitate plant growth by direct or indirect mechanisms. Direct promotion occurs when endophytes facilitate the acquisition of the environment resources including iron, nitrogen or phosphorous and when endophytes produce or regulate plant hormones. Indirect mechanisms of plant growth promotion consist in the protection of plant against nematodes or pathogenic bacteria and fungi by producing of antibiotics and cell wall-degrading enzymes. Moreover, endophytes produce a variety of metabolites, such as alkaloids, quinones, flavonoids, phenolic acids, steroids, peptides, terpenoids and antioxidant compounds, which protect plants against pathogens and grazing by animals [Tan Zou 2001, Strobel and Daisy 2003, Huang et al. 2007, Glick 2015].

The most common endophytic properties are production of phytohormones, nitrogen fixation and induction of resistance to plant pathogens by production of antibiotics. In this study we attempted to determine whether selected endophytic bacteria with strong antifungal activity against plant pathogens [Goryluk-Salmonowicz et al. 2016] produced indole-3-acetic acid (IAA). Moreover, we investigated the influence of seed dressing with these bacteria on seedling development of triticale. The root length, seed germination ability and germination energy were assayed according to Polish Standard.

## MATERIAL AND METHODS

### Endophytic strains

Bacterial endophytes used in this study were isolated from native growing herbs collected in Poland [Goryluk-Salmonowicz et al. 2016]. Five endophytic strains with the strongest antifungal activity against plant pathogens, were selected for the experiments: *Bacillus* sp. 30B (identified in this study), *Erwinia persicina* 2-5b (GenBank accession number KJ130483), *Pseudomonas azotoformans* P2, *P. azotoformans* P3 (GenBank accession numbers KJ130484 and KJ130485, respectively) and *P. cedrina* N2-1a (GenBank accession number KJ130486). Strains were isolated from the following plants: *Chelidonium majus* L. (30B, 2-5b), *Solidago gigantea* (N2-1a) and *Elymus repens* (P2, P3). All strains are deposited in collection of Department of Microbial Biology, at the Warsaw University of Life Sciences in Poland.

### Strain identification

Endophytic strains were identified based on morphological observation, biochemical properties and 16SrRNA gene analysis as previously described [Goryluk-Salmonowicz et al. 2016]. One strain, *Bacillus* sp. 30B, classified to *Bacillus subtilis* group needed further researches as 16SrRNA gene sequence, was too highly conserved to enable *Bacillus* species differentiation. 16SrRNA analysis (in the NCBI database using Standard Nucleotide BLAST) showed 100% sequence homology of 30B strain to *Bacillus amyloliquefaciens* and *Bacillus subtilis*

species [Goryluk-Salmonowicz et al. 2016]. One of the accurate method applied for differentiation between *B. subtilis* and *B. amyloliquefaciens* is randomly amplified polymorphic DNA analysis (RAPD). According to Ronimus et al. [1997], RAPD-PCR was performed using primer OPR13 (5'-GGACGACAAG-3') and the band pattern was compared with those obtained by González et al. [2013], who were working with isolates from *Bacillus subtilis* group, as well.

### IAA production and quantification

To determine the amounts of IAA produced by each bacterial strain, the Salkowski's method was used [Ehmann 1977]. Strains were grown in Luria Bertani broth (LB) without tryptophan or LB with 1mg ml<sup>-1</sup> of L-tryptophan. To inoculate the culture medium, 10 µl of bacteria in exponential phase were used. Cultures were incubated at 30°C for 10 days, in darkness on a rotary shaker (150 rpm). After 1, 7 and 10 days of incubation, the amount of IAA produced was measured using colorimetric technique. Each culture was centrifuged (10 000 rpm, 15 min) and 2 ml of supernatant was mixed with 3 drops of o-phosphoric acid and 4 ml of Salkowski's reagent (1 ml of 0.5 M FeCl<sub>3</sub> in 49 ml of 35% perchloric acid (HClO<sub>4</sub>)). Samples were vortexed, incubated for 25 minutes in darkness and their absorbance was read at 530 nm. The amounts of IAA were read using calibration curve made using IAA as a standard (1–100 µg·ml<sup>-1</sup>) [Gordon and Weber 1951].

### Seed coating with IAA producing endophytes – effect on root length

The experimental material comprised triticale seeds cv. “Kargo” (Plant Breeding in Strzelce, Poland). Before the experiment, seeds were surface sterilized using 95% ethanol and mercury chloride (0.2%) and washed with sterile distilled water several times. Then, seeds were soaked for 30 minutes in suspension of 1-day, 7-day or 10-day culture of bacteria grown in LB medium with or without tryptophan. Bacteria cultures were prepared the same way as for IAA production test (see above). Seeds coated in sterile water, sterile LB medium without Trp and sterile LB medium with Trp were also prepared as

controls. After coating, 15 seeds were placed in sterile plastic containers with tissue-paper soaked in sterile distilled water to obtain 65% of humidity. For every treatment method, three containers were prepared (45 seeds). Seeds were incubated for 5 days at 20°C and the root length of seedlings was measured (cm).

### Seed coating with sterile media and bacteria cultures – effect on seed germination

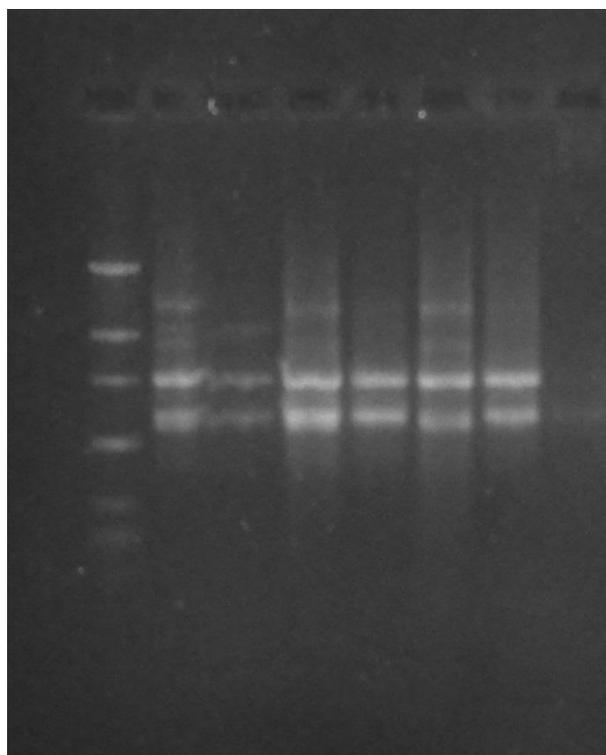
Firstly, the influence of sterile media necessary for preparing bacteria cultures, on seed germination was investigated. Before treatment, seeds were surface sterilized using 95% ethanol and mercury chloride (0.2%) and washed with sterile distilled water several times. Then, seeds were soaked in the following media: nutrient broth (NB), 0.01% NB, 3% molasses, 0.3% molasses, potato dextrose broth (PDB) and 10% PDB. Seeds treated with sterile distilled water were used as a control.

Seed germination was evaluated according to Polish Standard PN-R-65950:1994 (Sowing material). Seeds were placed in sterile plastic containers with tissue-paper soaked in sterile distilled water to obtain 65% of humidity. For every treatment method, three containers with 100 seeds were prepared. The experiment was performed at 20°C (+/-1°C). The number of germinated seeds was counted after 4 (germination energy) and 8 (germination ability) days of incubation. Results were analyzed according to PN-R-65950:1994 and the final result was expressed as arithmetic mean.

The best medium that did not have any negative influence on seed germination process was chosen to prepare bacteria cultures. Chosen medium was inoculated with one of the endophytic strain and incubated for 12 h at 30°C in rotary shaker (150 rpm). Then, seeds were dressed with bacterial cultures and the germination ability and energy were evaluated as described above.

### Statistical analysis

Data were analyzed using one-way and two-way analysis of variance. Homogenous groups of means were determined with the Tukey's procedure of multiple comparisons at the significance level 0.05. The analyses were performed using Statgraphics 4.1 statistical package.



**Fig. 1.** OPR13 RAPD profile of isolate 30B. Lane1, DNA molecular mass standard DNA Dramix 3794bp, 1639bp, 1191bp, 697bp, 718bp, 399bp (A&A Biotechnology); lanes 2, 4–7 30B isolate

## RESULTS AND DISCUSSION

Tested endophytic strains were analyzed regarding their IAA production ability and their influence on triticale seed germination and root development. RAPD profile analysis using OPR13 primer allowed for identification of *Bacillus* sp. 30B strain as *Bacillus amyloliquefaciens*. This strain shared identical band pattern as *B. amyloliquefaciens* isolates UY976, UY1091, T11, T144 analyzed by González et al. [2013]. In both cases, representative bands of 2300bp, 1250bp, and 850bp were present (RAPD profile, Fig. 1). Bacteria belonging to the *Bacillus* genera, are the most often isolated endophytes. These species of endophytes have been widely found in various medicinal plants. In 2012, Miller et al. isolated different bacterial endophytes including *Bacillus* species from Chinese medicinal plants, *Pinellia ter-*

*nata*, *Lycium chinense*, *Digitalis purpurea*, *Leonurus heterophyllus*, *Bletilla striata*, *Belamcanda chinensis* (L.), *Pinellia pedatisecta*, *Taxus yunnanensis*. Yuan et al. [2012] isolated, from *Ginkgo biloba* grown in China, a strain classified as *Bacillus amyloliquefaciens* CGMCC5569. Other researchers also isolated *Bacillus* strains from Chinese medicinal plant, *Lonicera japonica* [Zhao et al. 2015]. Furthermore, *Bacillus* endophytic strains were isolated from *Teucrium polium* L. medicinal plant grown in Egypt [Hassan 2017].

### IAA production in cultures

Strains were verified for IAA production using Salkowski reagent. All strains produced IAA while growing in LB medium without tryptophan (Trp) or LB medium supplemented with Trp (Tab. 2). Statistical analysis of results demonstrated that addition of Trp to the culture medium resulted in an increase in IAA content, as the average level of IAA produced by strains in LB medium was  $7.95 \mu\text{g} \cdot \text{ml}^{-1}$ , while the average level of IAA produced in the presence of Trp was  $19.21 \mu\text{g} \cdot \text{ml}^{-1}$  (difference was statistically important). Our results are in line with those reported by Hassan in 2017, who observed that the IAA production by endophytic bacteria increased with increasing

tryptophan concentration in the medium.

Also, the time of bacterial incubation had statistically important influence on the quantity of IAA produced. After 1 day of incubation, strains produced the lowest quantity of IAA: average  $5.27 \mu\text{g} \cdot \text{ml}^{-1}$ , after 7 days the level of IAA increased up to  $15.96 \mu\text{g} \cdot \text{ml}^{-1}$  (average) and after 10 days strains produced the highest IAA levels: average  $19.53 \mu\text{g} \cdot \text{ml}^{-1}$  of IAA. There were significantly different levels of IAA produced by strains. The highest levels of IAA were observed for *Pseudomonas azotoformans* P3 strain incubated for 10 days in LB medium with Trp –  $35.71 \mu\text{g} \cdot \text{ml}^{-1}$ . This IAA level was higher than IAA levels in cultures of the rest of tested strains incubated in LB medium and cultures incubated for 1 day in LB medium supplemented with tryptophan. High IAA levels were observed after 7 and 10 days cultures of *P. cedrina* N2-1a in LB medium with Trp ( $30.06 \mu\text{g} \cdot \text{ml}^{-1}$  and  $31.50 \mu\text{g} \cdot \text{ml}^{-1}$ , respectively). More than  $20 \mu\text{g} \cdot \text{ml}^{-1}$  were produced by *P. azotoformans* P2 and P3 strains and by *B. amyloliquefaciens* 30B strain incubated for 7 and 10 days in LB medium with Trp.

In conclusion, all five endophytic strains presented in this study were found to produce IAA that ranged from  $0.56$  to  $35.71 \mu\text{g} \cdot \text{ml}^{-1}$ . IAA production in

**Table 2.** Comparison of average IAA production ( $\mu\text{g} \cdot \text{ml}^{-1}$ ) by endophytic bacterial strains growing on LB medium supplemented or not with tryptophan, after 1, 7 and 10 days of incubation

Strain	Medium	Average IAA level ( $\mu\text{g} \cdot \text{ml}^{-1}$ )		
		1 day of incubation	7 days of incubation	10 days of incubation
<i>E. persicina</i> 2-5b	LB	0.56 a	5.02 abc	7.97 abcde
	LB + Trp	1.85 ab	13.50 bcdefg	13.51 bcdefg
<i>B. amyloliquefaciens</i> 30B	LB	1.26 ab	7.92 abcde	8.86 abcde
	LB + Trp	4.63 abc	21.92 fghi	24.54 ghij
<i>P. azotoformans</i> P2	LB	3.29 abc	8.56 abcde	9.74 abcdef
	LB + Trp	6.89 abcde	25.93 ghij	26.25 jkl
<i>P. azotoformans</i> P3	LB	0.61 a	5.93 abcd	17.92 defgh
	LB + Trp	3.81 abc	25.38 ghij	35.71 m
<i>P. cedrina</i> N2-1a	LB	6.80 abcde	15.43 cdefg	19.34 efghi
	LB + Trp	22.71 ghi	30.06 klm	31.5 lm

Letters indicate homogenous groups of means, which do not differ significantly at  $\alpha = 0.05$

the absence of tryptophan, which is an IAA precursor, was reported for all strains and ranged between 0.56 to 19.34  $\mu\text{g ml}^{-1}$  while addition of tryptophan to culture medium resulted in three-fold increase in the amount of IAA produced by strains (from 1.85 to 35.71  $\mu\text{g ml}^{-1}$ ). Our results seems to be very promising, while other researchers reported lower IAA levels produced by bacterial strains incubated in similar conditions. Uma Maheswari et al. [2013] isolated endophytic bacteria, that after seven days of incubation in LB medium without Trp, produced IAA from 0.05 to 0.12  $\mu\text{g ml}^{-1}$  (while our strains produced up to 15.43  $\mu\text{g ml}^{-1}$ ). Ngoma et al. [2014] reported endophytes that after two days of incubation in a medium without Trp produced from 0.16 to 2.2  $\mu\text{g ml}^{-1}$  IAA. Our strains, after one day of incubation, produced higher IAA levels, for example *P. cedrina* N2-1a produced 6.80  $\mu\text{g ml}^{-1}$ . The highest level of IAA was reported for *P. azotoformans* P3 strain that produced 35.71  $\mu\text{g ml}^{-1}$  after 10 days of incubation in medium supplemented with Trp. Similar studies were conducted by Ahmad et al. [2005]. After seven days of *Pseudomonas* sp. culture incubation in LB supplemented with tryptophan (1 mg  $\text{ml}^{-1}$ ), the researchers reported presence of IAA, though the amounts of IAA were lower in comparison to our results. Ahmad

reported bacteria producing from 10.4 to 28.3  $\mu\text{g IAA ml}^{-1}$  while our *Pseudomonas* sp. strains after seven days of incubation produced from 25.38 to 35.71  $\mu\text{g IAA ml}^{-1}$ . Ngoma et al. [2014] incubated endophytes for two days in medium with Trp (1 mg  $\text{ml}^{-1}$ ) and reported the presence of IAA within the range of 0.21–2.84  $\mu\text{g ml}^{-1}$  while our strains produced from 1.85 to 22.71  $\mu\text{g IAA ml}^{-1}$  after one day of incubation with Trp. It is noteworthy that the isolate producing the highest IAA levels, obtained by Ngoma et al. [2014], was classified to *Pseudomonas* species (MA39 strain), which is in accordance with our results (*Pseudomonas azotoformans* N2-1a strain).

#### The effect of seed treatment with bacterial cultures on triticale root development

It is well known that IAA has a positive influence on the growth and development of plants. The question was asked, if the effect of seed dressing with endophytic bacteria producing IAA has a positive influence on triticale root development. Before seeds were treated with bacterial suspensions, the effect of sterile culture media on root development was investigated. Seeds were soaked in distilled water, LB medium and LB medium supplemented with Trp.

**Table 3.** Influence of seed dressing with endophytic bacteria on triticale root length

Strain	Seed treatment		Average root length (cm)		
	H <sub>2</sub> O	medium	1-day culture	7-day culture	10-day culture
<i>E. persicina</i> 2-5b		LB	7.31 def	7.74 efg	7.23 def
		LB + Trp	4.57 ab	5.95 bcd	5.56 bc
<i>B. amyloliquefaciens</i> 30B		LB	3.69 a	7.64 efg	7.71 efg
		LB + Trp	8.73 efghi	10.11 ijkl	10.67 jkl
<i>P. azotoformans</i> P2		LB	9.22 ghij	9.11 ghij	9.68 hijk
		LB + Trp	7.59 defg	11.30 klm	11.45 lm
<i>P. azotoformans</i> P3		LB	8.26 efgh	8.66 efghi	7.14 cde
		LB + Trp	10.12 ijkl	10.11 ijkl	12.76 mn
<i>P. cedrina</i> N2-1a		LB	8.80 fghi	9.69 hijk	10.53 jkl
		LB + Trp	11.61 lmn	13.23 no	14.48 o

Letters indicate homogenous groups of means, which do not differ significantly at  $\alpha = 0.05$

After incubation, there were no significant differences between root length of seedlings treated with water and those treated with media. Afterwards, seeds were treated with bacterial cultures incubated for 1, 7 or 10 days in LB medium or LB medium supplemented with tryptophan. Analysis of results confirmed that seed bacterization had significant influence on triticale root length (Tab. 3). The best effect was observed for *Pseudomonas cedrina* N2-1a seed treatment. The longest roots were recorded after seed treatment with 10-days culture of *P. cedrina* in LB medium with Trp – 14.48 cm. Positive effect on root length was obtained also for seed treatment with other bacterial cultures incubated for 10 days in LB medium with Trp, where root length reached from 10.67 cm to 12.76 cm and for 10 days LB culture of *P. cedrina* N2-1a (10.53 cm). Very long roots were obtained also for seeds soaked with *P. azotoformans* P2 and *P. cedrina* N2-1a 7 days cultures in LB with Trp and for 24 h culture in LB with Trp and 10 days culture in LB of *P. cedrina* N2-1a strain (from 11.30 cm to 13.23 cm). On the other hand, negative effect on root length was observed after seed coating with *E. persicina* 2-5b strain incubated in LB medium supplemented with tryptophan and after application of 1 day culture of *B. amyloliquefaciens* grown in LB medium.

Concluding, analysis of the results allow for the statement that seed treatment with bacteria producing high IAA levels ( $>22 \mu\text{g ml}^{-1}$ ) have a positive influence on root development as 2 to 6 cm longer roots were obtained in comparison to control roots. The most beneficial effect on root growth of triticale seedlings was found for *Pseudomonas cedrina* N2-1a

strain. Similar results were obtained by other researchers, who have also observed that IAA producing endophytes positively affected the root development of agricultural plants. Hassan et al. [2017] observed that IAA producing endophytes used to inoculate the maize seeds increased the root length. It is noteworthy that some studies confirmed that after inoculation with endophytes of agricultural plants, the root length is comparable to the control seedlings or even shorter [Abbamondi et al. 2016].

#### The influence of seed bacterization on triticale seed germination

The purpose of the preliminary experiment was to select such a culture medium that did not have an additional effect on the germination of seeds. As the tested media had no influence on seed germination, 3% molasses solution was selected for bacterial cultures preparation. Germination ability and energy was slightly lower after seed treatment with *E. persicina* 2-5b strain, but the results were not statistically significant in comparison to control seeds (Tab. 4). Seed treatment with three *Pseudomonas* sp. strains had a positive influence on germination, but results did not differ significantly from those obtained for control seeds. The conclusion is that bacterial seed dressing using tested bacterial strains had no significant influence on seed germination.

Although no endophytic strain had positive influence on the seed germination ability and energy, the results can be considered very promising, because commercially available biopreparations used in crop protection often inhibit the germination of seeds as demonstrated by Horoszkiewicz-Janka and Jajor

**Table 4.** Effects of seed bacterization on triticale seed germination

Method of seed treatment	Germination ability (%)	Germination energy (%)
H <sub>2</sub> O	95.33 a	94.33 a
<i>E. persicina</i> 2-5b	92.66 a	91.66 a
<i>B. amyloliquefaciens</i> 30B	95.00 a	94.66 a
<i>P. azotoformans</i> P2	96.33 a	96.00 a
<i>P. azotoformans</i> P3	96.00 a	95.66 a
<i>P. cedrina</i> N2-1a	96.00 a	94.66 a

Letters indicate homogenous groups of means, which do not differ significantly at  $\alpha = 0.05$  in rows

[2007]. The researchers proved that Cedomon<sup>R</sup> containing *Pseudomonas chlororaphis* bacterial cells had negative effect on barley seed germination ability and energy. Since our strains had no negative effect on seed germination, they can be considered good candidates for plant growth biocontrol agents. Tested endophytic strains, especially *Pseudomonas* species, not only enhance the root development of triticale (present study), but also have fungistatic activity against plant pathogenic fungi [Goryluk-Salmonowicz et al. 2016]. It is known that endophytic contribution to plant growth and thereby crop productivity can be enhanced by application of plant growth promoting endophytes (PGPE). Chemical agents used in crop protection are expensive and have harmful impact on environment. Therefore, in the recent years, there has been an increased interest in searching for biological preparations containing non-pathogenic microorganisms. These preparations very often contain soil microorganisms. Application of endophytes give a spectrum of new possibilities. Our work suggests that endophytes belonging to *Pseudomonas* species have the most applicable potential. Further works conducted in the field conditions are necessary to explore the possibility to use endophytic strains described in this paper as biology control agents.

## CONCLUSIONS

The presented study has demonstrated that endophytic bacteria isolated from *Chelidonium majus* L, *Solidago gigantea* and *Elymus repens* produce IAA, which ranged from 0.56 to 35.71  $\mu\text{g ml}^{-1}$ . The highest IAA levels were produced by *Pseudomonas azotiformans* P3 endophytic strain isolated from *Elymus repens* (35.71  $\mu\text{g ml}^{-1}$ ). Furthermore, triticale seed treatment with endophytic bacteria producing high IAA levels ( $>22 \mu\text{g ml}^{-1}$ ) have a positive influence on root development. *Pseudomonas cedrina* N2-1a, endophytic strain isolated from *Solidago gigantea*, has the most beneficial effect on triticale root growth. Notably, the research shows that tested endophytic bacteria had no negative influence on the triticale seed germination ability and energy, while most

commercially available biopreparations for crop protection very often inhibit the seed germination.

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