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ORIGINAL ARTICLE

Tubercle disease of sugar beets, the lost pathogen, and the thin border between pathogenicity and stimulatory effect

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Abstract

Tubercle disease or a bacterial pocket disease of sugar beets are names used to describe one of the gall-malformed diseases of sugar beet roots. *Xanthomonas beticola* is the historical name of the pathogen supposedly causing bacterial pocket disease. There were no isolates deposited in any collection corresponding to the originally isolated bacteria, except two strains from the NCPPB (National Collection of Plant Pathogenic Bacteria, UK). However, both isolates were identified as related to *Bacillus pumilus*, which raised doubts about their pathogenicity. In our laboratory, greenhouse, and preliminary field experiments, we demonstrated that such strains are not pathogenic to sugar beets. Furthermore, both strains promoted their growth, improved their yield quality, and partly protected them against *Rhizoctonia solani* in a field experiment.

Keywords: bacterial pocket disease, IAA, plant growth promotion, sugar beet, tubercle disease

Introduction

In the past, *Xanthomonas beticola* was thought to be the causal agent of tubercle disease or bacterial pocket disease on sugar beet roots. The disease was observed for the first time about 100 years ago in the USA and, after that, it was recorded only occasionally, until 2014, when it was noticed in the central part of Poland. Symptoms of the disease include the development of tumor-like deformations located on the upper part of roots, although some deformations can also be observed below the root's head. Some infected roots show irregular galls on the whole root surface, while some show varying amounts of galls on the crown. The disease can cause a significant decrease in sugar content in malformed roots: 1–1.5% and up to 3–6% less sugar (Moliszewska *et al.* 2016, 2018) than healthy roots. A number of laboratories trying to isolate bacteria from diseased roots did not recover *X. beticola* (Moliszewska *et al.* 2016, 2018). A disease with symptoms similar to tubercle disease has been observed in Poland in the field, in 2014 and from 2015 to 2017 (Moliszewska *et al.* 2016, 2018). The disease also occurred in the territories of the Russian Federation and in Moldova, Georgia, Armenia, and Ukraine (Moliszewska *et al.* 2016). The first description of the disease caused by this bacterium was given by Serbinow in 1913, although symptoms of the disease were also denoted in 1911 (Brown 1928). It was reported in 1956 in Armenia (USSR) on table beets and artificially infected fodder and sugar beets (Galach'yan 1961). Starr and Stephens (1964) described the disease caused by *X. beticola* (strain n. ICPB XB l09Smr). Sherf and MacNab (1986) described *X. beticola* as causing tuberculosis of the table beet and sugar beets as well as fodder beets. Nyvall (1989) described bacterial pocket disease in the USA (Colorado, Maryland, Michigan, New Mexico, Utah, Virginia, Wisconsin, and Wyoming). *Xanthomonas beticola* also appeared in Ukraine, in 1984 and it was described by Kozyrovskaya *et al.* (1984). A description of the disease caused by *X. beticola* still exists in the Polish version of LIZ pages (LIZ 2023), and in the online manual of plant diseases (Lazarev 2003–2009). It may be also found in the Benada *et al.* (1984) manual

of sugar beet pests and diseases printed in Poland in 1984, and descriptions of a pocket disease are given by Draycott (2006) and Harveson *et al.* (2009).

The bacterial pocket disease has occasionally been observed since 1972 in New York State, showing less than 0.1% diseased roots in the whole root yield. However, in 1988, the disease was again observed in 10% of the beets in the same commercial fields. Searching for the pathogen, *Erwinia herbicola* was isolated from tumors of table beets from four fields located near Geneva, New York. This strain, labeled as EH112Y, was tumorigenic on beet slices and seedlings' roots. It was also tumorigenic on *Gypsophila paniculata*. Only strains obtained from tumors on mature roots or beet seedlings caused disease symptoms. The isolates resembling *E. herbicola* obtained from germinated seeds could not cause tumors on seedlings or on *G. paniculata* (Burr *et al.* 1991). Currently, APS Image Database includes pictures of bacterial pocket with *Pantoea agglomerans* pv. *betae* (syn. *Erwinia herbicola* pv. *betae*) as a causal agent. Responsibility for the pathogenicity comes from plasmid pPATH, which determines the ability to cause tumors (Burr *et al.* 1991; Weinthal *et al.* 2007).

Tumors developed on plants are mainly created by the additional production of plant hormones, typically by indole-3-acetic acid (IAA) and cytokinin overproduction. The level of IAA determines whether its producer acts as a pathogen or beneficial organism (Barash and Manulis-Sasson 2007; Moliszewska and Nabrdalik 2020). Pathogenic activity is associated with *hrp/hrc* gene cluster bound with plasmid-borne pathogenicity islands in addition to phytohormones IAA and cytokinin production. According to a model given by Barash and Manulis-Sasson (2007, 2009) for the emergence of new pathogens, we think that the reverse process is also possible. When phytohormones productivity is not lost and when it persists on a safe and stable level the producer may serve as a beneficial microorganism, changing its position from a pathogen to an effective microorganism. However, pathogenic properties develop due to the complex mechanism of various gene products. A dual behavior of avirulence and virulence coming from the *avr*/*vir* gene was recognized on the pPATH plasmid of *E. herbicola* pv. *gypsophilae* (currently *Pantoea agglomerans* pv. *gypsophilae*) (Ezra *et al.* 2000).

We obtained two isolates, presumably associated with the bacterial pocket disease of sugar beets, from the National Collection of Plant Pathogenic Bacteria, UK (NCPPB). The strains were stored as *Xanthomonas beticola*, strain 1927 and *X. beticola*, strain 1831. To the best of our knowledge, they were the only isolates named *X. beticola* in the world, although both had a note of uncertain status. Therefore, we decided to carry out preliminary experiments to check the potential pathogenicity of both isolates of *X. beticola* from the NCPPB collection.

Materials and Methods

Bacteria

Two isolates of *X. beticola,* strain 1927 and strain 1831, obtained from the NCPPB collection in 2016, were used. They were added to the NCPPB collection in 1966. Originally, they were included in The International Collection of Phytopathogenic Bacteria (ICPB; Department of Bacteriology, University of California, Davis, California, 95616, USA) with their original codes ICPB XB4 and ICPB XB5. The strain *X. beticola* 1831 was better documented. It was isolated from sugar beets in 1926 in the USA by H.A. Elcock. Since then, they have been freeze-dried in glass ampoules and stored at 4°C (personal information kindly provided by Ms. Charlotte Howard (Microbiology Culture Collections Assistant of NCPPB). They were further classified (March 13, 2016) using the fatty acid methyl ester analysis (FAME) by NCPPB staff. FAME showed that both strains were taxonomically close to *Bacillus pumilus,* although they were still stored in NCPPB as pathogenic *Xanthomonas beticola* strains. Due to the uncertain status of both bacteria, for the purpose of this research, we used the name for the tested bacteria strain Bp 1831 and strain Bp 1927.

DNA isolation and amplification

DNA was extracted from 1-day old liquid cultures with the use of the GeneMATRIX Bacterial & Yeast Genomic DNA Purification Kit (EURx, Poland) following the manufacturer's instructions. The weight of the cell pellet did not exceed 50 mg per single minicolumn. DNA samples were stored at –20°C until analysis. The amplification was carried out with the following primers related to the HV region of the 16S rRNA gene and designed for the identification or grouping of *Bacillus* species: 47F (5'-GCCTAATACATGCAAGTCGAGCG-3') and 365R (5'-ACTGCTGCCTCCCGTAGGAGT-3') (Goto *et al.* 2000) and with the use of Color Taq PCR Master Mix (2x) (EURx, Poland). PCR reactions were conducted in 50 µl capacity each, with 5 µl of template DNA (from 1-day-old cultures), following the protocol of the manufacturer. Cycling conditions for PCR included: initial denaturation at 94°C for 3 min; 35 cycles of denaturation at 94°C for 30 sec, annealing at 54°C for 1 min, and elongation at 72°C for 1 min. The final cycle was followed by extension at 72°C for 7 min. The amplified products were separated by electrophoresis with a 1% agarose gel and stained with SimplySafe (EURx).

PCR samples were sequenced by Genomed (www.genomed.pl). Amplicon sizes were 289 and 294 base pairs for the strain Bp1927 and Bp 1831, respectively. Sequence identities were determined with the National Center for Biotechnology Information BLAST tool (www.ncbi.nlm.nih.gov/BLAST/).

Phylogenetic analysis

Phylogenetic analysis was done by constructing a phylogenetic tree. The tree was inferred by the Maximum Likelihood method and Tamura-Nei model (Tamura and Nei 1993). The bootstrap consensus tree was inferred from 1000 replicates. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model and then selecting the topology with superior log likelihood value. There were 310 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar *et al.* 2018).

Inoculum preparation

Bacterial inocula were prepared on LB (Laurin-Bertani) liquid medium 1 day before using them in greenhouse and field experiments. Flasks containing 750 ml of LB medium were inoculated with 1 ml of the pure culture and then incubated for 24 hours at 37°C on a rotary shaker. The final density of cultures was 10^8 colony forming units per ml (cfu · ml^{-1}).

Greenhouse test

The greenhouse test was conducted in a facility belonging to a breeding company (Kutnowska Hodowla Buraka Cukrowego – KHBC) using pots of five liters capacity, filled with steam-sterilized mixed peat and soil (1 : 1). Tests consisted of three combinations, each in three replications: 1 – combination infected with strain Bp 1831, 2 – combination infected with strain Bp 1927, 3 – control. For each combination, we used three pots, each sown with commercially prepared sugar beet seeds cv. Janosik, to obtain three healthy and well-developed plants in each pot. After 8 weeks, each plant was treated with 5 ml of bacterial inoculum, which was infused directly into the soil around the plant. In control pots, a sterile LB medium was used and applied in the same way. All pots were watered using a drip irrigation system. They were fertilized monthly using Biohumus (Agrecol) – a liquid organic fertilizer. Plants were grown for 28 weeks and, then, harvested. At harvest, we checked the physiological and phytosanitary status of the plants. Roots and leaves were weighed separately. Roots were then analyzed to measure the total sugar content (%) and the content of K, Na, and N in the form of α-amino-N (mval · 100 g^{-1}). All tested chemical parameters are routinely used in sugar beet yield qualification by sugar beet industries and breeding companies. Chemical analyses were made using the automated system Venema in KHBC, Poland.

IAA production

The tested bacterial strains were basically known as tumorigenic bacteria, so we expected that a possible way of producing tumors was the ability to synthesize plant hormones, especially IAA, which also plays an important role in plant endophytic bacteria (Moliszewska and Nabrdalik 2020). To determine the amounts of IAA produced by tested bacterial strains, a colorimetric technique with the Salkowski reagent was applied (Szkop *et al.* 2012). Both isolates were grown in LB medium supplemented with L-tryptophan at concentrations of 0.1 and 1.0% and incubated at 30°C for 7 days. In control trials, the bacterial cultures were grown in a medium without L-tryptophan. The production of IAA was measured every 24 hours. After incubation, the cells were centrifuged (10,000 rpm for 20 minutes at 10°C) and 2 ml of supernatant was mixed with 4 ml of Salkowski's reagent and kept in the dark at room temperature. The optical density (OD) was measured at a wavelength of 530 nm after 30 minutes. The quantification of IAA was carried out using a standard curve with known concentrations of pure IAA (Sigma–Aldrich), prepared separately (Nabrdalik *et al.* 2018).

Field experiment

The field experiment was conducted in the KHBC area. The greenhouse experiment showed the nonpathogenic status of both tested bacteria and also potential for improvement of sugar beet growth and development. Therefore, in the next step we decided to check the possible protective ability of both tested bacteria. The field, chosen for this experiment, was infected in previous years by *Rhizoctonia solani* AG 2-2IIIB, which is pathogenic to sugar beets. As a result the assessment could show us the potential protective activity of the tested bacteria against one of the most important sugar beet pathogens. The tested combinations were arranged in the same way as in the greenhouse experiment. Each combination consisted of micro-plots arranged as single rows with 30 plants in each with four replications. Seeds of sugar beet cv. Janka were sown at the beginning of April in rows with a distance of 12 cm among seeds in rows Nine weeks after sowing (BBCH 17-19), each sugar beet plant was treated with 5 ml of bacterial suspension (density = 10^8 cfu · ml^{-1}),

which was prepared and used in the same way as the inoculum for the greenhouse test. At harvest, we checked the health of plant roots. Then they were weighed and analyzed chemically using the automated system Venema in KHBC, Poland. The results of the health condition of roots were counted as a percentage of diseased ones and calculated to obtain the infection coefficient (Ip), giving information on the disease severity, according to the Townsend-Heuberger formula (Nabrdalik *et al.* 2018).

Statistical analysis

Statistical analysis of the results' significance was determined using an analysis of variance (ANOVA) followed by Tukey's HSD test. Values were considered significantly different at $p < 0.05$. The statistical calculations were done with a Microsoft Excel spreadsheet.

Results

Searching the most comparable sequences of 16S RNA in the GeneBank database using the BLAST tool did not allow for unequivocal determination of the taxonomic status of both tested bacteria and confirmed that they are not related to *P. agglomerans pv. betae*. However, it is clear that both sequences are almost identical and they differ at only three positions and three deletions for the strain Bp 1927. The sequence comparison in GeneBank and phylogenetic analysis made it possible to assign both of them to the genus *Bacillus* (Fig. 1), showing only some relationships to *B. pumilus* (identity 99.29%). The HV region, analyzed in this research, is highly conserved within the species and allows for determining the *Bacillus* species. The HV regions of our isolates differed from the *B. pumilus* group, however, they were the most similar to this group.

Greenhouse test

The greenhouse test results clearly showed that neither tested isolate possessed any pathogenic properties under greenhouse conditions. Sugar beets grown in pots inoculated by strain Bp 1831 showed greater mass than the controls, indicating growth stimulation. Strain Bp 1927 apparently stimulated the growth of sugar beets as well, but the data were not statistically significant. An analysis of the average mass of root yield obtained in one pot, clearly and significantly showed that both strains can improve the mass of roots and the mass of leaves (Table 1). In comparison to the untreated control, chemical analysis showed a greater sugar content (18.33 – 19.14%) with low molassegenic compounds (K, Na, and α-amino-N) in roots treated by both bacteria. Strain Bp 1831 slightly reduced α-amino-N content, but the result was not statistically significant (Table 2).

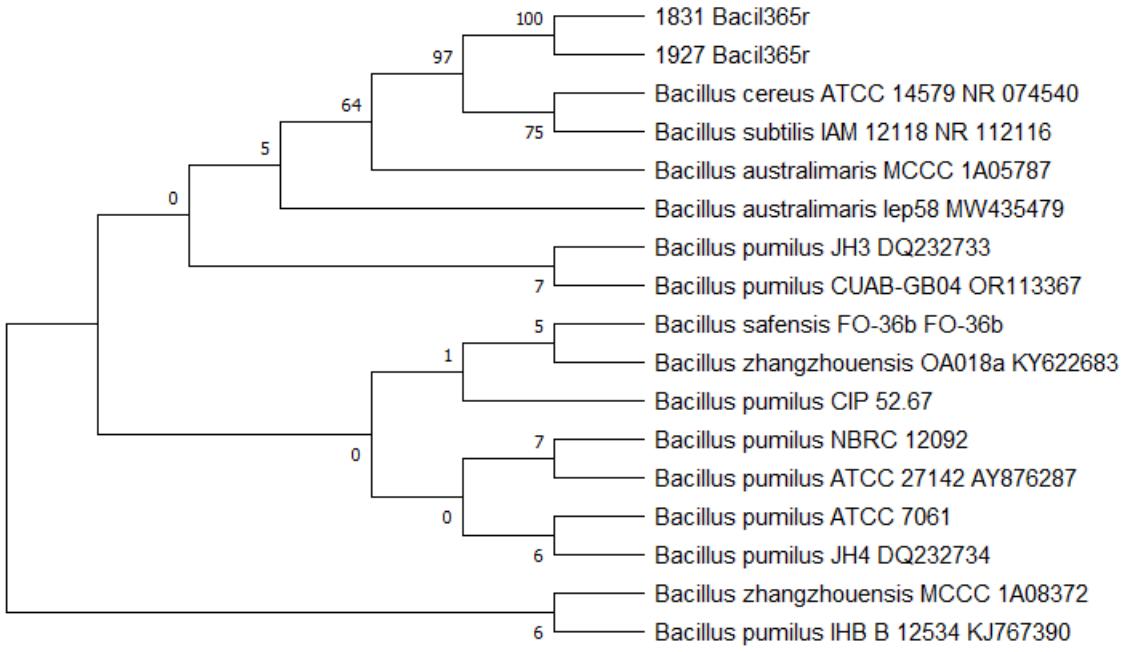

Fig. 1. Phylogenetic relationships among tested bacteria and other *Bacillus* strains. The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model (Strain Bp 1831 assigned as 1831Bacil365r; strain Bp 1927 assigned as 1927Bacil365r)

Table 1. Influence of tested bacterial strains on the yield of sugar beet in the greenhouse experiment ($p < 0.05$)

Bacterial strain	Weight of one plant [g]	Weight of roots per pot [g]	Weight of leaves per pot [g]
Strain Bp 1831	74.89 ± 24.41*	108.36 ± 33.51*	80.56 ± 25.20*
Strain Bp 1927	53.76 ± 18.73	85.31 ± 26.52*	75.96 ± 30.81*
Control	47.34 ± 27.56	64.87 ± 18.86	49.83 ± 27.16

*results significantly different from the control

Table 2. Influence of tested bacterial strains on sugar beet roots quality in the greenhouse experiment

Bacterial strain	Sugar [%]	K [mval ·100 g^{-1}]	Na [mval ·100 g^{-1}]	α-amino-N content [mval ·100 g^{-1}]
Strain Bp 1831	19.14 ± 0.08**	21.30 ± 0.00**	7.43 ± 0.06*	10.93 ± 0.55
Strain Bp 1927	18.33 ± 0.04**	23.73 ± 0.71**	7.87 ± 0.15 *	7.10 ± 3.21*
Control	17.93 ± 0.04**	28.63 ± 0.15**	9.17± 0.23	14.00 ± 0.69**
Strain Bp 1831 (% of control)	106.75	74.40	81.03	78.07
Strain Bp 1927 (% of control)	102.23	82.89	85.82	50.71

*results significant at $p < 0.05$, **results significant at $p < 0.01$ (in columns)

Field experiment

Results showed reduced severity of *R. solani* infection after inoculating the soil with both strains Bp 1831 and Bp 1927. Under the influence of tested bacteria, the severity of infection by *R. solani* measured according to the "infection coefficient" (Ip) was approx. 34.6–39.0%. When not protected by bacteria control the Ip was higher – 65.2% (untreated by tested strains) (Fig. 2). The percentage of infected roots treated by both strains was not significantly different in comparison to the control (Fig. 2). The quality of yield was not distinctly improved, but we noticed a slight tendency of both strains to positively influence the root quality in the field test (Table 3).

We did not observe tubercle disease symptoms on any beets treated with either bacterial isolate, neither in the greenhouse nor in the field experiment. However, in previous years, in the same field, the tubercle disease of sugar beet roots was observed.

Treating sugar beets with tested bacteria revealed a significant positive correlation coefficient between the presence of both bacteria and sugar content in roots, for strain Bp 1831 – $r = 0.72$ and for Strain 1927 – $r = 0.70$, which indicates and confirms the

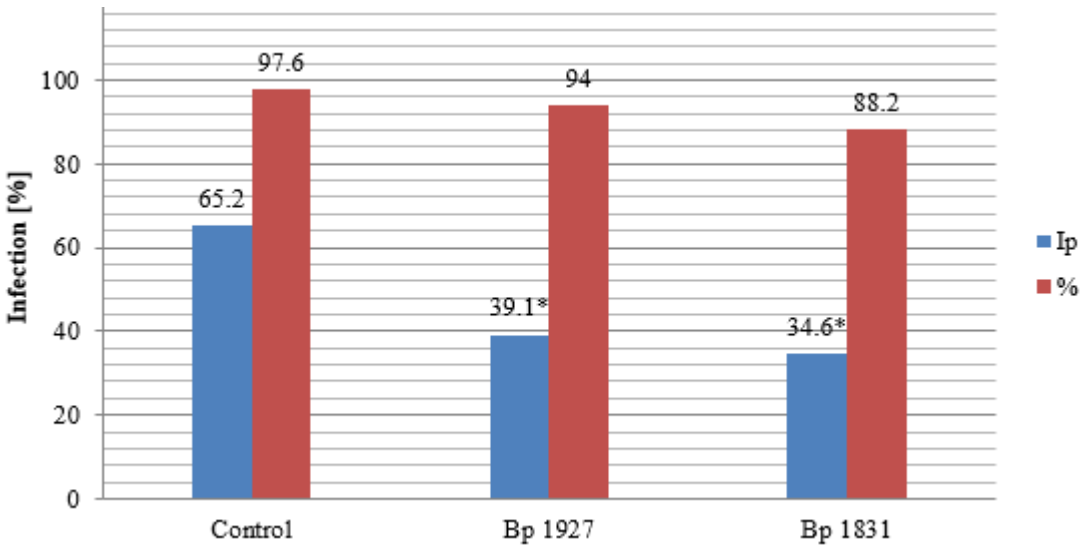

Fig. 2. Influence of tested strains on the health of sugar beet roots in the field experiment; *result significantly different from the control at $p < 0.05$ (Ip – disease coefficient; % – percent of diseased roots)

Table 3. Influence of tested bacterial strains on the yield quality of sugar beet in the field experiment

Bacterial strain	Sugar [%]	K [mval ·100 g^{-1}]	Na [mval ·100 g^{-1}]	α-amino-N content [mval ·100 g^{-1}]
Strain Bp 1831	14.48 ± 0.40	35.00 ± 0.85	8.10 ± 0.10	16.50 ± 3.22
Strain Bp 1927	13.91 ± 0.83	37.95 ± 3.43	7.70 ± 1.68	18.68 ± 2.17
Control	14.32 ± 1.68	37.70 ± 3.60	7.08 ± 1.08	17.15 ± 2.79

positive influence of both bacteria on sugar production in the greenhouse experiment. This kind of result obtained in the field experiment was less effective. For strain Bp 1831 the correlation coefficient was $r = 0.52$ and we did not obtained such result for strain Bp 1927, the correlation coefficient was $r = 0.26$.

IAA production

Both tested strains showed the ability to produce IAA in LB medium, at 30°C and pH 7. The amount of IAA increased with time and pure LB medium consisted of almost 1 μg · ml^{-1} after 120 h of incubation for strain Bp 1927 and after 144 h for strain Bp 1831 (Fig. 3). Under the same conditions, but with an amendment of L-tryptophan (0.1 and 1.0%), both strains produced a greater amount of IAA, which was dependent on the L-tryptophan amount and time of incubation. The maximum amount of IAA production, 9.4 μg · ml^{-1}, was obtained after 168 h for Strain 1927 in LB + 1.0% of L-tryptophan, and it was eight times more than in pure LB medium and almost four times more than in LB + 0.1% of L-tryptophan. The strain Bp 1831 produced less of the active compound in LB + 1.0% of L-tryptophan, and it was about six times more than in the pure control medium and 2.3 times more than in medium with lower L-tryptophan addition (Figs. 3–4). Strain Bp 1831 isolate showed a time-independent production of IAA in the medium with the addition of 1.0% of L-tryptophan, while a lower addition of L-tryptophan demonstrated a time dependence of IAA production.

Discussion

The tested strains were originally deposited as *X. beticola*, including one of them with confirmed pathogenic properties. Both were identified by NCPPB staff as related to *B. pumilus*. In the molecular analysis, this was partially confirmed, although they were the most comparable to each other and also showed some similarity to other strains of the *Bacillus* clade, e.g., *B. australimaris* (Fig. 1) (Liu *et al.* 2016). At this level of genomic analysis there is not enough information to

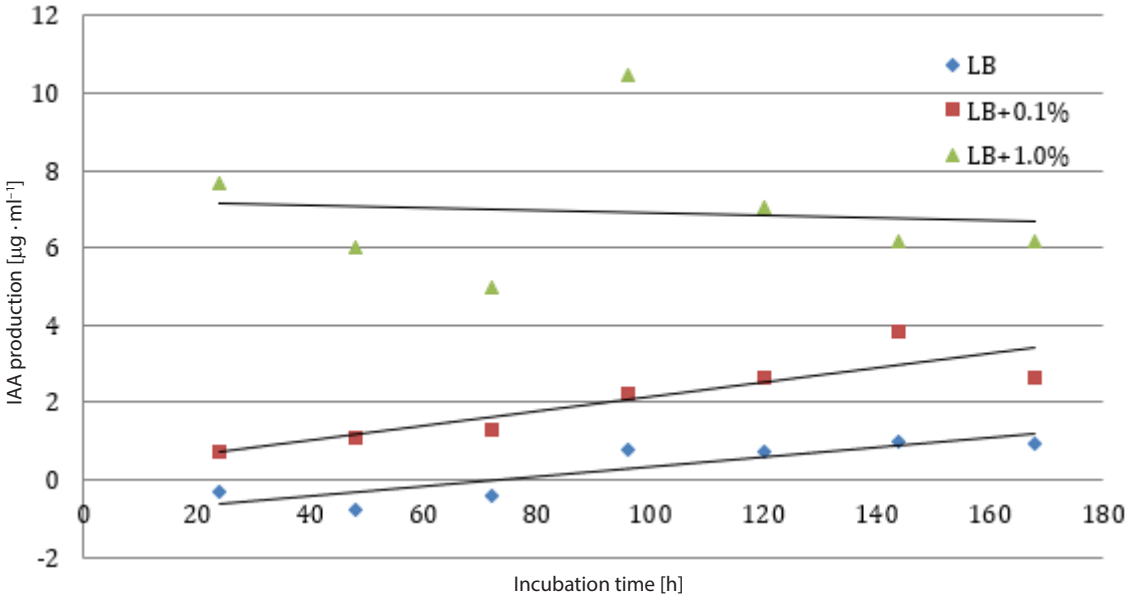

Fig. 3. Influence of time and addition of L-tryptophan on the IAA productivity by the strain Bp 1831. Results are significantly different from the control (LB) at $p < 0.05$ – data were analyzed statistically for the first and last measurements (LB + 0.1%, LB + 1.0% – means the LB medium with 0.1% and 1.0% addition of L-tryptophan, respectively)

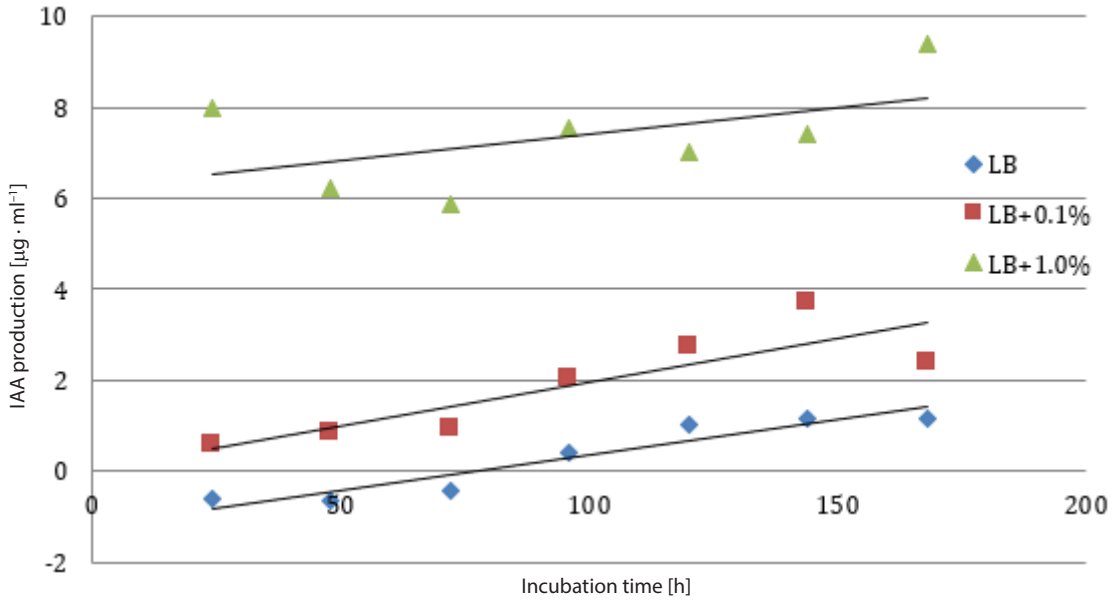

Fig. 4. Influence of time and addition of L-tryptophan on the IAA productivity by the strain Bp 1927. Results are significantly different from the control (LB) at $p < 0.05$ – data were analyzed statistically for the first and last measurements (LB + 0.1%, LB + 1.0% – means the LB medium with 0.1% and 1.0% addition of L-tryptophan, respectively)

determine the species status of both bacteria, however, the molecular analysis was based on the highly specific HV region for *Bacillus* species (Vandamme *et al.* 1996; Goto *et al.* 2000). Additionally, molecular comparison (BLAST tool) found that the tested strains Bp 1831 and Bp 1927 were not related to *P. agglomerans* pv. *betae*, presently known as a causal agent of sugar beet tubercle disease. In the past, there were many difficulties in the proper identification of the true causal agent of the bacterial pocket disease, known also as tubercle disease. Burr *et al.* (1991) and Weinthal *et al.* (2007) found that the disease is caused by *P. agglomerans* pv. *betae* (*E. herbicola* pv. *betae*), and tumors developed because of pathogenic genes carried on the plasmid pPHAT. Weinthal *et al.* (2007) and Manulis *et al.* (1998) reported similar observations. In the research carried out by Burr *et al.* (1991), there was no correlation between the ability to produce IAA-resembling compounds and the possibility of causing tumors; nontumorigenic strains produced equal amounts of IAA or more (Burr *et al.* 1991). Both strains (Bp 1831 and Bp 1927) tested in our research showed the ability to produce auxins, and the production was higher when they were supplemented by an additional amount of L-tryptophan. The level of IAA production was almost the same for both, although strain Bp 1831 demonstrated stable synthesis of the compound when supplemented with a higher dose (1%) of precursor molecule. Supplementation of the medium with precursors always yields better production of IAA by microorganisms with such ability (Moliszewska and Nabrdalik 2020). The IAA produced by nonpathogenic strains usually promotes plant growth (Patil 2019). Patil (2019) identified *B. amyloliquefaciens* and *Brevundimonas diminuta* as strains with a high capability of promoting plant growth by producing IAA and other plant growth-promoting compounds, such as siderophores, ammonia, catalase, and hydrogen cyanide. Bacteria of the genus *Bacillus* are the most common strains with plant growth-promoting ability. Other genera include *Pseudomonas*, *Azospirillum, Bradyrhizobium,* and *Pantoea*. Among *Bacillus* species, *B. amyloliquefaciens* and *B. subtilis* are more frequently mentioned than *B. pumilus*, although the latter has the potential to act as a PGPR. One possible positive role of PGPR may be to protect plants against disease (Moliszewska and Nabrdalik 2020). The infection coefficient (Ip) for roots treated with strain Bp 1831 or Bp 1927 was lower than in the unprotected control. This suggests that both bacterial strains may be protective agents against *R. solani*. However, they reduced the severity of infection, but not the percentage of infected roots. It was clearly shown by Enya *et al.* (2007a) that in the *Pantoea* genus, 90% of strains are able to protect plants against fungal diseases, in contrast to the genus *Bacillus,* in which *B. pumilus* comprised 38% of *Bacillus* strains (Enya *et al.* 2007b). Thus, Bacilli do not seem to be the most potent ones in plant protection against fungal diseases. *Pantoea agglomerans* BC17 and BC45 tested against *R. solani* in laboratory experiments showed potential as protective agents. The isolates inhibited the growth of mycelium directly on the

medium supplemented with bacterial metabolites and decreased disease development on sugar beet seedlings in a pot test. The isolate BC17 had better protective and inhibitory activity (Nabrdalik *et al.* 2018). The pathogenicity of *P. agglomerans* pv. *betae* in addition to these results demonstrate the broad possible activity of different isolates of the *P. agglomerans* complex, which is presumably controlled by the presence or absence of pPATH.

Results obtained for both tested strains (Bp 1831, Bp 1927), originally deposited as pathogens of sugar beets, clearly did not have pathogenic capability. They acted as promoters of sugar beet growth in the greenhouse experiment, increasing the mass and yield quality (more sugar and less molassogenic compound) of plants treated with bacterial cultures. Comparable results were obtained in the field experiment, although in the field infected with *R. solani* we observed a decrease in disease severity with no clear improvement in root chemical quality. The inhibitory effect of *B. pumilus* (strain 7 km) on the take-all disease (*Gaeumannomyces graminis* var. *tritici*) of wheat might be related to its ability to enhance defense responses in wheat roots (Sari *et al.* 2007). Similar results were obtained for *B. pumilus* C9, which significantly promoted *Arabidopsis thaliana* growth under greenhouse conditions, as well as protected those plants against *Pseudomonas syringae* DC3000. The C9 *Bacillus* strain was also able to promote the growth of tomato and corn plants, producing greater fresh and dry mass, which resemble our greenhouse results. Additionally, it increased the tomato biomass under poor nutrient conditions and nitrogen supplementation. The mode of action of its protective role was its induction of the ISR genes (Huang *et al.* 2014).

In the experiments of Chinese researchers, published in 2013, the supernatant of *B. pumilus* SQR-N43 showed a direct antifungal activity against *R. solani*, which caused cucumber damping-off. The inhibitory activity of *Bacillus* isolate was due to antibiotic production. It was isolated from crude culture and then semi-purified using the ammonium sulfate precipitation method. Its molecular weight was between 500 and 1000 kDa. Both crude cultures as well as semi-purified antibiotics affected fungal activity, and antibiotic activity was increased proportionally to the culture density, although activity was lost when the culture achieved the stationary phase (Huang *et al.* 2013). In our experiment, we did not check the ability of strains Bp 1831 and Bp 1927 to produce antibiotics, so we cannot exclude this possibility.

In conclusion, strains Bp 1831 and Bp 1927 demonstrated clearly non-pathogenic features, as well as potentially promoting and protective activity for sugar beet plants. These results also confirm our conclusion contained in the article "Tubercle disease…" (Moliszewska *et al.* 2016, 2018) that *X. beticola* does not exist in the form described at the beginning of the XX-th century. Furthermore, they do not resemble *P. agglomerans* pv. *betae*, the currently recognized causal agent of tubercle disease of sugar beet, as demonstrated by molecular analyses. When it was seen that both strains did not show any pathogenic properties they were excluded from the NCPPB collection of plant pathogenic bacteria, which definitely closes the career of *X. beticola* in science.

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