Bacteria Isolated from the Aeration Chamber of Wastewater Treatment Plants Used in the Biocontrol and Promotion of Wheat Growth

Sebastian Wojciech Przemieniecki 1,* , Anna Gorczyca 2 , Ewelina Matras 2 , Krzysztof Krawczyk 3 , Jędrzej Mastalerz 1 and Arkadiusz Zakrzewski 4

1 Department of Entomology, Phytopathology and Molecular Diagnostics, University of Warmia and Mazury in Olsztyn, Prawocheńskiego 17, 10-720 Olsztyn, Poland; jendrekmasta@wp.pl
2 Department of Microbiology and Biomonitoring, University of Agriculture in Krakow, Mickiewicza 21, 31-120 Krakow, Poland; rrgorczy@cyf-kr.edu.pl (A.G.); ewelina.matras1@poczta.onet.pl (E.M.)
3 Department of Molecular Biology and Biotechnology, Institute of Plant Protection-National Research Institute, Władysława Węgorka 20, 60-318 Poznań, Poland; k.krawczyk222@gmail.com
4 Department of Industrial and Food Microbiology, University of Warmia and Mazury, Plac Cieszyński 1, 10-726 Olsztyn, Poland; arkadiusz.zakrzewski@uwm.edu.pl

* Correspondence: sebastian.przemieniecki@uwm.edu.pl; Tel.: +48-895233597

Received: 5 October 2020; Accepted: 3 November 2020; Published: 16 November 2020

Abstract: Background: Antagonisms against Fusarium spp. and multi-trait to protect and improve fertilization of wheat by bacterial strains from activated sludge were assessed. Methods: Isolated strains obtained were identified by 16S rRNA gene sequencing and the MALDI-TOF method, and their enzymatic profile was investigated. Treated plant growth-promoting bacteria (PGPB) wheat kernels were grown in pots with soil contaminated with Fusarium conidia. Activated sludge is a collection of microorganisms exposed to strong environmental pressure (chemicals) and antagonistic properties. Results: The isolated bacterial strains were similar to: Ps-1 (Serratia liquefaciens), Ps-15 (Serratia sp.) and Ps-9 (Pseudomonas helleri). The dual culture assay showed the highest antagonism of Ps-9 vs. Fusarium spp. The tested bacteria showed activity in the production of chitinase, a variety of proteases, enzymes that degrade various sugars, and esterase, which creates a complex that allows for a variety of strategies to control phytopathogens. The Ps-9 strain was able to solubilize phosphate. The Ps-9 and Ps-15 strains showed good ammonification ability. A marked improvement was observed in test variants in pots inoculated with Fusarium spores after the use of Ps-9. The Ps-9 strain reduced the disease index to traces of symptoms of both species of Fusarium and increased the grain weight. Conclusions: The Ps-9 strain was proven to have high potential for application in the biocontrol and promotion of wheat growth.

Keywords: activated sludge; Fusarium spp., PGPB; Pseudomonas helleri; Serratia sp.

1. Introduction

The use of plant growth-promoting bacteria (PGPB) is a highly promising method of integrated plant protection [1–3]. In the future, PGPB are expected to be used in support of chemical fertilizers, pesticides, and artificial growth regulators to help reduce doses and to decrease numerous side effects on sustainable agriculture. Among PGPB, the most numerous group are bacteria of the genera Acinetobacter, Pseudomonas (e.g., P. aeruginosa, P. aureofaciens, P. fluorescens, P. marginalis, P. putida), in addition to Bacillus (e.g., B. cereus, B. subtilis, B. coagulans, B. laterosporus, B. megaterium, B. mycoides, B. pasteurii, B. sphaericus), Enterobacter (E. agglomerans, E. cloaca), Azotobacter, Azospirillum, Burkholderia, Pantoea, Rhizobium, and Bradyrhizobium [3,4]. There is extensive published research indicating that bacteria have...
a positive effect on the growth and development of plants [5–7]. The positive impact of microorganisms on higher plants is a result of supplying them with the necessary minerals (i.e., by facilitating nitrogen uptake, dissolving phosphorus compounds) [6,8]. In addition, these bacteria produce phytohormones (auxins, gibberellins, cytokinins) and improve the soil structure. Plant vaccination with the genus *Pseudomonas* has also been shown to increase plant yield by 144% and alleviate the stress caused by abiotic factors [8–10].

PGPB can also be used for plant protection. Numerous PGPB bacteria are known for their antagonistic activity against pathogenic bacteria and fungi. They compete for an ecological niche and nutrients (including sugars and amino acids). They produce antibiotics or other compounds that inhibit the growth of phytopathogens. These include ammonia, butyrolactone, 2,4-diacetylchloroglucine (DAPG), hydrogen cyanide (HCN), phenazine-1-carboxylic acid (PCA), pyoluteorin (PLT), pyrrolnitrin (PRN), viscosinamide, kanosamine, oligomycin A, oomycin A, xanthobaccin A, and zwittermicin A [8,11–14]. Another example is the binding of soil iron by bacterial siderophores, which makes it inaccessible to pathogenic fungi, thus limiting their growth [15,16].

Many studies focus on biocontrol of the most pervasive and burdensome phytopathogenic fungi, such as *Fusarium* spp. Pathogenic fungi adapt quickly to conditions in the environment and demonstrate great tolerance to environmental changes, which affect numerous agricultural plant species. Pathogenic fungi cause significant economic losses due to a decrease in the quality and quantity of crops. The losses of cereals resulting from their presence can range from 7% to 70%. These fungi infect plants at various stages of development, causing many diseases, such as fusariosis and stem diseases. *Fusarium* fungi produce mycotoxins (i.e., deoxynivalenol, nivalenol, zearalenone, fumonisins, vomitoxin, T2) which are dangerous for humans, animals, plants, and microorganisms [17–19]. Moreover, *Fusarium* fungi may exist as endophytic fungi and cause asymptomatic infection or live as a saprobiont, which is probably the defense mechanism which facilitates their spread and allows them to adapt to unfavorable environmental conditions [20].

Wastewater management is currently a global problem. Research into the agricultural use of wastewater is primarily concerned with the potential for fertilization. Due to an increase in the level of organic matter and nutrients in soil, irrigation of crops with wastewater may be used as a method of conventional fertilization [21,22]. The cultivation under these conditions of crops that are tolerant to salts and accumulate heavy metals provides a solution to the problem of waste disposal [23]. Nonetheless, the cultivation of sensitive species fertilized with untreated wastewater causes severe stress which limits the growth and fertility of plants, and thus is not recommended [24,25]. A relatively new area of research is to consider wastewater as a source of effective biofertilizers, that is, of microorganisms promoting plant growth [26–30].

In this work, an activated sludge was examined because it is an example of a microbial community exposed to changing environmental conditions and constant pressure of various chemical substances [31]. In our previous work [20], we proved the usefulness of bacteria obtained from wastewater treatment plants for biological protection against phytopathogens. Based on those results, we isolated and described PGPB strains which are resistant to environmental conditions while also showing a strong antagonistic effect against phytopathogenic fungi.

The aim of this study was to evaluate the antagonistic properties of three PGPB strains from activated sludge against *F. culmorum* and *F. solani*, and to characterize their potential for application in crop production by assessing the ability of PGPB strains to promote growth and development of spring wheat in the presence of *F. culmorum* and *F. solani.**

2. Materials and Methods

2.1. Sampling

Samples of activated sludge were taken directly from an aeration chamber of the Biskupiec Wastewater Treatment Plant (53°50'52.8'' N, 20°55'55.2'' E). In the laboratory, the samples were shaken
Subsequently, a decimal dilution of 1 mL of the tested samples in 9 mL of sterile saline (0.85% NaCl) solution was performed and aliquots of 0.1 mL of the sample were transferred into TSA (Tryptic Soy Agar Medium, Merck, Germany). All morphological types were collected and stored at −80 °C for future analyses.

2.2. Phylogenetic and MALDI-TOF Analysis

First, total DNA was extracted from pure culture using a Genomic Mini AX Bacteria Spin kit (A&A Biotechnology, Poland). Bacterial strains were identified based on the 16S rRNA nucleotide sequences obtained by using the primer pair 27F and 1492R designed by Lane [32] with PCR conditions as described in previous work [33]. Sequencing was performed at the company Genomed (Poland). All obtained sequences were aligned and consensus sequences with a length of 1000 bp were verified against the NCBI-GenBank database (National Center of Biotechnology Information, USA) using the BLAST tool. Next, the phylogenetic tree was constructed using the Neighbor-Joining method. The dendrogram included 16S rRNA sequences of the tested strains and other bacterial strains obtained from the Genbank database. For alignment and tree construction, the MEGA6 program was used. The sequences were deposited in the GenBank database under the following accession numbers: MK404730, MK404731, and MK404732.

The MALDI-TOF method was used to confirm the results of identification. Measurements were taken using a VitekMS MALDI-TOF (bioMérieux, Marcy l’Etoile, France) with an acceleration voltage of 200 kV, a mass range of 2–20 kDa, a laser frequency of 50 Hz, and an extraction delay time of 200 ns. All mass fingerprints were analyzed against the VitekMS V2.0 research use-only (RUO; SARAMIS version 4.13) databases (bioMérieux, Marcy l’Etoile, France). Isolates were identified in duplicate using the direct transfer protocol according to the manufacturer’s recommendations. The isolates were briefly cultured for 48 h at 30 °C on TSA (Merck, Germany) and were then transferred to the target plate. One microliter of MALDI matrix VitekMS-CHCA was added to the spots. After crystallization of the matrix solution, the target was loaded into the MALDI-TOF MS system.

2.3. Antagonistic Test

The inhibition of pathogenic fungi by PGPB strains was analyzed with the method described by Przemieniecki et al. [33]. Mycelial discs with a diameter of 5 mm were cut from cultures of F. culmorum or F. solani and located on the center part of the PDA medium (BD Difco, USA) in Petri dishes. The analyzed bacteria were cultured on both sides next to disc, at a distance of 3 cm. Incubation was performed for 72 h at 28 °C (optimal for saprotrophic microorganisms), after which the percentage of inhibition was calculated as follows:

\[
\% \text{ inhibition} = \left[1 - \frac{\text{area of mycelium in presence of PGPB}}{\text{area of control mycelium}}\right] \times 100 (1)
\]

2.4. Enzymatic Activity

Biochemical parameters were evaluated in qualitative analyses using API® 20NE and API® ZYM kits (Biomérieux, France) according to the applicable instructions. Tests were incubated at the optimal temperature for saprotrophic bacteria.

2.5. Pot Experiment

Greenhouse tests were performed in round black plastic pots. These pots, of 28 cm diameter and 22 cm height, were filled with 8 kg of thoroughly mixed soils (loamy sand, pH\textsubscript{KCl} 6.0, total nitrogen −0.2%, P\textsubscript{2}O\textsubscript{5} −24.8 mg 100 g\textsuperscript{−1} of soil, K\textsubscript{2}O −20.1 mg 100 g\textsuperscript{−1} of soil, magnesium −4.8 mg 100 g\textsuperscript{−1} of soil). Prior to planting, soil water content was adjusted to a 60% field capacity in all pots. Before sowing, seeds were treated with 1% carboxymethyl cellulose (CMC) solution containing 5·10\textsuperscript{8} CFU·mL\textsuperscript{−1} of the Ps-1, Ps-9, and Ps-15 strains. Seeds treated with sterile water were used
as the experiment control (blank). Phytopathogens macroconidia (*F. culmorum* and *F. solani*) were obtained from a PDA medium (Potato Dextrose Agar, Difco, USA) pure culture by elution with sterile demineralized water. The reference strains were obtained from the UWM collection. The concentration of conidia was estimated by OD (optical density) measurement at a wavelength of 600 nm (Nanodrop 2000C, Thermo Fisher Scientific, Waltham, MA, USA). For treatments including the presence of phytopathogens, the 50 mL suspension of conidia was added to the pot cultivation layer of soil (~25 cm). Next, the soil was mixed and the final concentration of conidia in the fresh mass of soil was assessed as $10^5$ g$^{-1}$. At the end of this process, the experimental soils samples were: (1) the control, without input of macroconidia; and soil contaminated with (2) *F. culmorum* and (3) *F. solani* macroconidia as negative controls. Twenty-four control seeds and seeds of spring wheat cv. Bombona treated with the bacteria strains Ps-1, Ps-9, and Ps-15 were sown in pots with experimental soils samples (1, 2, 3) at a depth of 1 cm. Each experimental variant contained 3 pots with 8 plants. Throughout the entire period of vegetation of the wheat, optimal humidity conditions were maintained, using the same quantity of irrigation in each pot. The cultivation was carried out in an unheated greenhouse that was maintained as close as possible to field conditions. The spring wheat plants were harvested at the BBCH89 stage (full maturity of grain). Ear and stalk length were measured manually. Individual ears were cut. Roots were carefully washed to remove soil using tap water to reduce root loss and their length was measured manually. Excess moisture on the roots was blotted on bibulous papers before measuring the fresh weight of roots. Fresh weights of ears and roots were measured on an electronic analytical scale (Radwag, model AS 220.R2, [d]: 0.1 mg) at an ambient temperature of 21 °C. Then, the samples were dried in a laboratory oven (Memmert, model UNE 200–800) at a continuous temperature of 70 °C for 72 h until constant weight was reached. Dried ears and root samples were weighed once more and the percentage of dry matter was calculated (dry sample weight/fresh sample weight). Individual ears were threshed out and grain weights per ear were examined to determine yields.

The Disease Index (DI) was determined according to a 5-point scale, from 0 for no symptoms of the disease to 4 for the strongest symptoms, and then presented on a percentage scale. The results are expressed as a DI in percent, calculated based on the formula according Liu and Liu [34]:

$$DI = \frac{\Sigma (a \cdot b)}{n \cdot i} \cdot 100\%$$  

where:

$\Sigma (a \cdot b)$—the sum of the products of the degree of scale and the number of plants infected to this degree;

$n$—total number of analyzed plants;

$i$—the highest degree of scale.

2.6. Statistical Calculations

First, the normality of the distribution of the obtained results was tested (Shapiro-Wilk test). The antagonism potential (inhibitions zones) and biometry were calculated by ANOVA ($p = 0.05$) with the Duncan test in Statistica 12 (StatSoft, Tulsa, OK, USA). PCA (principal component analysis) and AHC (agglomerative hierarchical clustering) were performed in XLSTAT software (Addinsoft, https://www.xlstat.com/). AHC involving whole biometrical parameters was calculated using Bray and Curtis dissimilarity with the Ward method, while AHC used for the comparison of enzymatic properties was calculated by Euclidean distance based on UPGMA.

3. Results

3.1. Identification of Analyzed Strains

The result of phylogenetic analysis is shown in Figure 1. Three strains of bacteria used in this study were identified (at species level) based on the 16S rRNA sequence for two of the three
isolates. A small subunit of ribosomal sequences (16S) was 98% similar to *Serratia liquefaciens* for Ps-1, 98% similar to *Pseudomonas helleri* for Ps-9, and 99% similar to *Serratia grimesii* or *S. quinivorans* for Ps-15. The MALDI-TOF analyses confirmed phylogenetic results and taxonomic membership for all three strains.

![Figure 1](image.png)

**Figure 1.** A Neighbor-Joining phylogenetic tree based on 16S rRNA partial sequences demonstrated for the analyzed strains of plant growth-promoting bacteria (PGPB) compared to other strains; the bar indicates sequence divergence.

### 3.2. Antagonistic Test

The dual culture assay showed that the isolated PGPB strains are antagonists of the analyzed *F. culmorum* and *F. solani* species (Table 1). The highest *Fusarium* inhibition was recorded for the Ps-9 strain (Figure 2). The lowest inhibition was estimated for *F. solani* at 43% when treated with the Ps-1 strain (Table 1). The results show that the tested bacterial strains have the potential to be effective biological control agents.

| Pathogen    | F. culmorum | F. solani |
|-------------|-------------|-----------|
| PGPB Strain | Ps-1        | Ps-9      | Ps-15   |
| % inhibition| 50.23 cd    | 68.37 A   | 63.00 ab| 43.53 d | 66.47 A   | 56.73 bc |

* Means marked by the same letters are not statistically different according to the Duncan test (*p* = 0.05—small letter, *p* = 0.01—capital letter).
Table 1. Antagonistic properties of isolated bacteria strains tested against Fusarium spp. cultures.

| Pathogen | F. culmorum | F. solani |
|----------|-------------|-----------|
| PGPB Strain | Ps-1 | Ps-9 | Ps-15 | Ps-1 | Ps-9 | Ps-15 |
| % inhibition | 50.23 cd* | 68.37 A | 63.00 ab | 43.53 d | 66.47 A | 56.73 bc |

* Means marked by the same letters are not statistically different according to the Duncan test ($p = 0.05$—small letter, $p = 0.01$—capital letter).

Figure 2. Growth inhibition of Fusarium spp. strains caused by the Ps-9 strain using the dual culture method.

3.3. Enzymatic Activity

All three analyzed strains showed enzymatic activity for 15 tested enzymes and were identical in their qualitative appearance (Table 2). The strains differed in the range of assimilation of simple compounds. The Ps-1 strain was able to use substrates, whereas for the other two strains there was a differing level of assimilation in two cases (Ps-1 used D-glucose and trisodium citrate to a lesser extent). Among the six analyzed metabolic traits, the Ps-9 strain was most active. It was also observed that all bacterial strains were characterized by the activity of: β-galactosidase; α-glucosidase; N-acetyl-β-glucosaminidase (chitinase); proteases (such as leucine, valine, and trypsin arylamidase); lipases and short- and long-chain esterases; and the ability to use sugars and simple compounds, such as D-mannose, D-mannitol, N-acetylglucosamine, D-maltose, and assimilation of potassium gluconate. The Ps-9 and Ps-15 strains demonstrated a good ability to utilize D-glucose. The Ps-1 strain showed a poor ability to assimilate D-glucose and L-arabinose. All strains were characterized by low β-glucosidase (cellulase) activity. The strains showed a high ability for the assimilation of decanoic and malic organic acids. It was also observed that the strains expressed high abilities of denitrification and glucose fermentation. The Ps-9 strain showed good phosphate dissolution capacity. Ps-9 and Ps-15 strains showed good ammonification ability and the Ps-1 strain had poor ammonification ability. None of the tested bacterial strains showed the ability to produce indole (Table 2).
Table 2. Biochemical parameters of tested Ps-1, Ps-9, and Ps-15 bacteria strains.

| Biochemical Properties | Strain |
|------------------------|--------|
|                        | Ps-1   | Ps-9   | Ps-15  |
| Enzymatic activity     |        |        |        |
| Alkaline phosphatase   | +      | +      | +      |
| Acid phosphatase       | +      | +      | +      |
| Esterase (C 4)         | +      | +      | +      |
| Ester lipase (C 8)     | +      | +      | +      |
| Lipase (C 14)          | ±      | ±      | +      |
| Leucine arylamidase    | +      | +      | +      |
| Valine arylamidase     | +      | +      | +      |
| Cystine arylamidase    | ±      | ±      | ±      |
| Trypsin                | +      | +      | +      |
| α-Chymotrypsin         | −      | −      | −      |
| Naphthol-AS-BI phosphohydrolase | +      | +      | +      |
| α-Galactosidase        | −      | −      | −      |
| β-Galactosidase        | +      | +      | +      |
| β-Glucuronidase        | −      | −      | −      |
| α-Glucosidase          | +      | +      | +      |
| β-Glucosidase          | ±      | ±      | ±      |
| N-Acetyl-β-glucosaminidase | +      | +      | +      |
| α-Mannosidase          | −      | −      | −      |
| α-Fructosidase         | −      | −      | −      |
| Arginine dihydrolase   | −      | −      | −      |
| Urease                 | −      | −      | −      |
| Protease               | +      | +      | +      |
| D-Glucose              | ±      | ±      | +      |
| L-Arabinose            | -      | -      | -      |
| D-Manose               | +      | +      | +      |
| D-Mannitol             | +      | +      | +      |
| N-Acetylglucosamine    | +      | +      | +      |
| D-Maltose              | +      | +      | +      |
| Assimilation           |        |        |        |
| Potassium gluconate    | +      | +      | +      |
| Capric acid            | +      | +      | +      |
| Adipic acid            | −      | −      | −      |
| Malic acid             | +      | +      | +      |
| Trisodium citrate      | ±      | ±      | +      |
| Phenylacetic acid      | −      | −      | −      |
| Reduction of nitrate to nitrite | +      | +      | +      |
| Reduction of nitrate to nitrogen | −      | −      | −      |
| Indole production      | −      | −      | −      |
| D-Glucose fermentation | +      | +      | +      |
| P-solubilization       | −      | +      | −      |
| Ammonification         | ±      | +      | +      |

(+) positive result, (±) weak enzymatic activity, (−) negative result.

3.4. Pot Experiment

The influence of the tested bacterial strains on the biometry and Disease Index (DI) of spring wheat is shown in Table 3. The grain yields varied. For both species of Fusarium, soil inoculation resulted in a significant decrease in the grain yield compared to the control. Additionally, the F. solani species had a significant harmful effect on all of the biometric parameters of plants, except for the dry mass of roots. The treatment of seeds with the Ps-9 strain provided effective protection against both F. culmorum and F. solani, as a result of which the grain yield was significantly higher than in the control. For the Ps-1 and Ps-15 strains, no significant improvement in grain yield was achieved as compared to the control, but the grain weight in these objects was greater than that of the plants grown in soil.
inoculated with phytopathogens (negative controls). Above all, however, bacterial inoculation showed high protective potential against diseases caused by fungi of the tested *Fusarium* genus. All strains of bacteria significantly reduced the occurrence of disease symptoms on the analyzed wheat plants, with the Ps-9 strains showing the greatest potential as an antagonist of phytopathogenic fungi of both species of *Fusarium*.

**Table 3.** Biometry and Disease Index (DI) of spring wheat plants cultivated from seeds treated with the examined strains (Ps-1, Ps-9, and Ps-15) and in soil inoculated with *F. culmorum* and *F. solani* macroconidia.

| Parameter                        | Control | *F. culmorum* | *F. solani* |
|----------------------------------|---------|----------------|-------------|
|                                  | None    | Ps-1           | Ps-9        | None    | Ps-1           | Ps-9        |
| Grain weight per ear [g]         | 2.65 cd | 2.44 e         | 2.51 de     | 3.27 a  | 2.68 cd        | 2.35 e      | 2.72 c  | 3 b   | 2.67 cd |
| Ear length [cm]                  | 4.32 b  | 4.17 b         | 4.31 ab     | 4.89 a  | 4.64 a         | 4.22 c      | 5.69 a  | 5.05 b | 4.34 b–d |
| Ear weight [g]                   | 3.50 b  | 2.84 c         | 3.68 ab     | 4.10 a  | 3.39 b         | 2.96 c      | 3.71 ab | 3.33 b | 3.75 ab |
| Ear dry mass [%]                 | 88.43 ab| 89.25 ab       | 90.11 a     | 88.86 ab| 89.32 ab       | 87.69 b     | 89.42 ab| 89.94 a| 89.59 ab|
| Stalk length [cm]                | 49.04 a | 47.25 a        | 47.59 a     | 46.59 a | 49.25 a        | 45.97 b     | 45.52 b| 45.82 b| 45.38 b |
| Root length [cm]                 | 7.76 cd | 6.98 c         | 8.34 bc     | 9.2 a   | 7.68 cd        | 7.26 d      | 11.7 ab | 12.38 A| 13.2 A  |
| Root weight [g]                  | 1.60 a  | 1.56 ab        | 1.92 a      | 1.76 a  | 1.64 a         | 1.39 b      | 2.22 a  | 1.88 a | 2.11 a  |
| Root dry mass [%]                | 34.33 b | 41.57 ab       | 43.79 a     | 43.99 a | 44.80 a        | 44.52 a     | 44.37 a | 44.1 a | 45.40 a |
| Disease Index (DI) [%]           | 24.1 ef | 68.5 b         | 51.9 cd     | 5.6 g   | 33.3 ef        | 92.6 A      | 55.6 bc | 5.6 g  | 37.0 de |

Total number of wheat plants in objects 24. Means marked by the same letters are not statistically different according to the Duncan test (*p* = 0.05—small letters, *p* = 0.01—capital letters/compared to control).

The calculated PCA (Figure 3) covered 70% of the total variability of the results, which shows the high power of the performed test. The results of the analysis indicate almost total separation of control objects (blank and negative) from objects treated with antagonistic bacteria. Variants without the use of any of the microorganisms showed a negative relation to the first and second coordinate axes, and were the closest to the Ps-15 + *F. culmorum* variant located around the center of the graph. Moreover, these objects were directly related to the largest stalk length values. In contrast, a group of proper control objects that were treated with *F. culmorum* or *F. solani* formed a pair of apparently different objects from the remainder of the results, which was accompanied by a closely related high DI index. Both Ps-1 and Ps-15 with *F. solani* variants showed a positive ratio towards both coordinate axes, and their location was mainly influenced by root dry mass, ear dry mass, root weight, and ear and root length, which indicates the direct impact of these beneficial bacteria on the improvement of these biometric parameters. However, the Ps-9 variants were similar to each other and relatively similar to the other objects using bacterial strain treatment. Their use had the greatest impact on ear weight, grain weight, and root length. In this analysis, a relationship between features was also observed, i.e., DI was almost completely negatively correlated with grain weight, root dry mass, and stalk weight. The other observed features were (at least partially) positively correlated with each other.

Agglomerative hierarchical clustering (AHC) analysis allowed the similarity in enzymatic activity between the analyzed groups of variants to be determined. It was observed that the strain Ps-9 differed clearly from Ps-1 and Ps-15 bacteria strains (Figure 4). Detailed analysis of the variants (PGPB bacteria × *Fusarium* spp.) allowed three different groups to be selected. Negative controls with both *Fusarium* pathogens formed one group, although they were nevertheless relatively similar to the group formed by the Ps-1 and Ps-15 PGPB strains and *F. culmorum* (Figure 4).
Figure 3. Principal component analysis (PCA) analysis of biometry and health parameters. Abbreviations: C—control; Fcul—*F. culmorum*; Fsol—*F. solani*; PS1, PS9, PS15—tested PGPB strains; DI—Disease Index.

Figure 4. Agglomerative hierarchical clustering indicating, on the right: enzymatic differences between PGPB strains (calculation based on results from Table 2); on the left: differences between variants used in pot tests (calculation based on results from Table 3). Abbreviations: Cont, C—control without the use of microorganisms; Fcul—*F. culmorum*; Fsol—*F. solani*; PS1, PS9, PS15—PGPB strains.

4. Discussions

In this study, the identified bacterial strains belonged to different species. The *S. liquefaciens* Ps-1 strain and the closely related *Serratia* sp. Ps-15 strain showed a high polymorphism of the 16S rRNA
sequence with respect to the well-known *S. marcescens* and *S. fonticola*. A similar relationship was observed between the *Pseudomonas helleri* Ps-9 sequence and *P. fluorescens*, *P. putida*, and *P. aeruginosa*. Moreover, the strain used in the study formed its own unique clade and was very closely related to *P. weihenstephanensis*. It can be presumed that the activated sludge environment is a reservoir of new species of bacteria that can significantly affect the growth of plants, especially under the pressure of phytopathogenic fungi [1,3,5,6]. The identified bacteria belonged to the *Pseudomonas* and *Serratia* genera, which is in accordance with our previous results [35]. The review by Mahmood et al. [36] of the role of PGPB in seed biopriming points to *Pseudomonas* and *Serratia* as having the greatest potential as inducers of resistance to biotic stress. Our results indicate that *Pseudomonas* bacteria are more useful than *Serratia* bacteria in the control of *Fusarium* in wheat, but the antagonistic properties of the two *Serratia* strains were also different. The high potential of *P. putida* as a PGPB and biological agent has been demonstrated by Ahemad and Khan [37] and Przemieniecki et al. [38]. Furthermore, similarly to other species of *Pseudomonas*, the *P. luteola* SP0113 strain inhibited the growth of *Fusarium* sp. [39]. *Fusarium* play a significant role in agriculture because they adversely affect cereals by infecting the seedlings which leads to major economic losses in yields [40]. Indirect harmfulness manifested in the contamination of grain yields by mycotoxins produced by *Fusarium* is also of huge importance for the global economy and human health [17]. In the case of other crops and phytopathogens, the *Serratia* strain can show higher potential. Abuamsha et al. [41], who tested seed treatment with *Serratia plymuthica* HRO-C48 and *Pseudomonas chlororaphis* MA 342 against *Leptosphaeria maculans*, showed a reduction in blackleg disease symptoms in oilseed rape of about 72 and 54% respectively. This indicates the need for targeted PGPB selection depending on the crop and the purpose of protection.

When analyzing the results presented for metabolism diversity, a small variability of traits between strains was observed. The most important features are the ability to produce chitinase (N-acetyl-β-glucosaminidase), a variety of proteases and enzymes that break down bonds in various sugars, and the enzymes that hydrolyze phosphate monoesterase, lipolytic and esterase bonds, creating a complex of multi-trait properties that allows for a variety of strategies to fight against phytopathogenic fungi. The above features in combination with phosphate solubilization (Ps-9) and ammonification enable efficient decomposition and assimilation of organic matter, which results in the provision of nutrients to plants [6,8,16,42]. Przemieniecki et al. [39] have also shown the high enzymatic activity and assimilation efficiency of the *Pseudomonas* sp. SP0113 strain. The *Pseudomonas* sp. SP0113 strain demonstrated the ability of denitrification and ammonification, allowing nitrogen to be taken up by the plant root system. Nitrogen plays a key role in yield creation. It was shown that a PGPB, which participates in all changes and nitrogen fixation, has a positive effect on plant growth [43,44]. The observed activity of alkaline phosphatase, acid phosphatase and other enzymes involved in the metabolism of phosphorus showed that the analyzed Ps-9 strain also increased the availability of organic phosphorus for plant roots. The use of PGPB strains generally improved the majority of biometric parameters only in plants growing in soil with *F. solani*. In the case of *F. culmorum*, such an improvement was observed after the use of the Ps-9 strain and it was the only strain which almost completely reduced the rate of disease intensity in both objects of the experiment. In addition, the most important factor was the increase in the most important indicator, i.e., the grain weight. Data obtained on the basis of PCA proved to be of the greatest help in the interpretation of the results because it made it possible to determine the relationships between the variants and the observed features, which was also confirmed to a lesser extent with the use of post hoc analysis. There was no significant impact of the Ps-15 strain on variants infected with *F. culmorum*, whereas there was found to be an excellent improvement in biometric parameters with most strains and a direct link between the grain weight and DJ, and thus the intensity of the plant disease state. An interesting, but not fully explained dependence was the result indicating that the higher the dry weight of the roots, the smaller the plant height. This indicates the uniqueness of the Ps-9 strain, which coincides overall with the best results in ANOVA and PCA analysis. A more detailed analysis of AHC revealed that, in global terms, the use of the Ps-1 and Ps-15 strains against *F. culmorum* may not be satisfactory, whereas against *F. solani*,
the difference in results (indicating improved biometric parameters) was high. Clearly, AHC analysis made it possible to determine only the Ps-9 strain to be highly effective as a biological agent against both species of *Fusarium*.

Satisfactory results were also obtained in our own previous works [39,45,46], which used the *P. luteola* SP0113 strain characterized by the control of phytopathogens of the genus *Fusarium* and a number of traits of PGPB, particularly P-solubilization. The beneficial effect of water bacteria on agricultural plants was presented in the paper by Goswami et al. [42] concerning the *Pseudomonas* sp. strain OG. The plant growth-promoting potential of *Pseudomonas* sp. OG strain was shown on Pokovskaya’s broth. A medium inoculated with *Pseudomonas* sp. OG had a growth-promoting effect resulting in high yield biometrics of chickpea and green gram plants.

Sludge disposal is an increasing environmental problem and few methods exist for sludge disposal and recycling. Recently, emphasis has been placed on the bioconversion of sludge into value-added products such as biopesticides, enzymes, bioplastics, and growth medium for PGPR as legume inoculants [47]. We have demonstrated the additional utility of sewage sludge, which can be a source of effective PGPB.

In previous studies [38], it was shown that bacteria isolated from the air, emitted by sewage treatment plants, can contribute to comparable protection against *Fusarium* spp. to that of soil bacteria. Among the several species belonging to *Advenella*, *Enterobacter*, *Lactococcus*, *Proteus*, *Pseudomonas*, and *Staphylococcus*, the *P. putida* showed the greatest positive effect on the reduction of *F. culmorum* and *F. graminearum*, both in plate and soil tests, and improved biometric parameters for control without the use of microorganisms. On the basis of the current results of principal component and AHC analysis, we showed there to be a weak antagonistic impact of the Ps-1 and Ps-15 strains on *F. culmorum*, whereas in the case of *F. solani*, the strains improved biometric parameters, i.e., root dry mass, ear dry mass, root weight, ear length, and root length. However, the Ps-9 strain proved to be highly effective against the two *Fusarium* species tested, which indicates the need for selection before introducing the strain for practical use in the method of biological promotion and protection of plants.

5. Conclusions

It can be concluded that plant growth-promoting potential is also expressed by non-agricultural bacterial strains, although this fact is not yet well-known to a broader audience. More study is needed in this area. Our results and the data from the available literature indicate that the tested strains may be effective in organic crops, because they show high resistance to a wide range of plant pathogens and in most cases positively affect plant growth.

Our experiment shows that the strains of bacteria used derived from activated sludge have excellent antagonistic properties against *Fusarium* spp. These PGPB demonstrate a wide range of biochemical activities that are useful in preventing the spread of plant pathogens. Moreover, they have an indirect impact on plant growth under more favorable environmental conditions. In summary, the strains used have the potential to be applied as a universal biological control agent, particularly the most effective strain *Pseudomonas* sp. Ps-9. To fully understand the mechanism of PGP action, future work should focus on genomic and proteomic analysis (next generation sequencing) of different antagonistic PGPB to detect the genes responsible for controlling phytopathogenic fungi.

**Author Contributions:** Conceptualization, S.W.P. and A.G.; methodology, S.W.P. and A.G.; software, S.W.P. and E.M.; validation, S.W.P., A.G., E.M. and K.K.; formal analysis, S.W.P., K.K., J.M. and A.Z.; investigation, S.W.P. and A.G.; resources, S.W.P. and A.G.; data curation, S.W.P. and A.G.; writing—original draft preparation, S.W.P. and A.G.; writing—review and editing, A.G., E.M., K.K., J.M. and A.Z.; visualization, S.W.P.; supervision, S.W.P. and A.G.; project administration, S.W.P.; funding acquisition, S.W.P. All authors have read and agreed to the published version of the manuscript.

**Funding:** This study was financed by the Ministry of Science and Higher Education, Republic of Poland No. 20.610.016–110.

**Acknowledgments:** The authors would like to thank Beata Duch and Karol Korzekwa for collecting samples from the wastewater treatment plant in Biskupiec and Samuel Pink for proofread the English language of the work.
Conflicts of Interest: The authors declare no conflict of interest.

References

1. Beneduzi, A.; Ambrosini, A.; Passaglia, L.M. Plant growth-promoting rhizobacteria (PGPR): Their potential as antagonists and biocontrol agents. *Genet. Mol. Biol.* 2012, 35, 1044–1051. [CrossRef] [PubMed]
2. Mendes, R.; Kruijt, M.; de Bruijn, I.; Dekkers, E.; van der Voort, M.; Schneider, J.H.; Piceno, Y.M.; DeSantis, T.Z.; Andersen, G.L.; Bakker, P.A.; et al. Deciphering the rhizosphere microbiome for disease-suppressive bacteria. *Science* 2011, 332, 1097–1100. [CrossRef] [PubMed]
3. Ahemad, M.; Kibret, M. Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. *J. King Saud Univ. Sci.* 2014, 26, 1–20. [CrossRef]
4. Bhattacharyya, P.N.; Jha, D.K. Plant growth-promoting rhizobacteria (PGPR): Emergence in agriculture. *World J. Microbiol. Biotechnol.* 2012, 28, 1327–1350. [CrossRef]
5. Hayat, R.; Ali, S.; Amara, U.; Khalid, R.; Ahmed, I. Soil beneficial bacteria and their role in plant growth promotion: A review. *Ann. Microbiol.* 2010, 60, 579–598. [CrossRef]
6. Khan, S.; Afzal, M.; Iqbal, S.; Khan, Q.M. Plant-bacteria partnerships for the remediation of hydrocarbon contaminated soils. *Chemosphere* 2013, 90, 1317–1332. [CrossRef]
7. Santoyo, G.; Moreno-Hagelsieb, G.; Orozco-Mosqueda Mdel, C.; Glick, B.R. Plant growth-promoting bacterial endophytes. *Microbiol. Res.* 2016, 183, 92–99. [CrossRef] [PubMed]
8. Przemieniecki, S.W.; Damszel, M.; Kurowski, T.P.; Mastalerz, J.; Kotlarz, K. Identification, ecological evaluation and phylogenetic analysis of non-symbiotic endophytic fungi colonizing timothy grass and perennial ryegrass grown in adjacent plots. *Grass Forage Sci.* 2019, 74, 42–52. [CrossRef]
21. Mojid, M.A.; Hossain, A.B.M.Z.; Wyseure, C.L. Impacts of municipal wastewater on basic soil properties as evaluated by soil column leaching experiment in laboratory. *J. Soil Sci. Plant Nutr.* 2019, 19, 402–412. [CrossRef]

22. Eid, E.M.; Hussain, A.A.; Taher, M.A.; Galal, T.M.; Shaltout, K.H.; Sewelam, N. Sewage sludge application enhances the growth of *Corchorus olitorius* plants and provides a sustainable practice for nutrient recirculation in agricultural soils. *J. Soil Sci. Plant Nutr.* 2020, 20, 149–159. [CrossRef]

23. Khalilzadeh, R.; Pirzad, A.; Sepehr, E.; Anwar, S. Long-Term effect of heavy metal–polluted wastewater irrigation on physiological and ecological parameters of *Salicornia europaea* L. *J. Soil Sci. Plant Nutr.* 2020, 20, 1574–1587. [CrossRef]

24. Liu, W.; Zhao, J.; Ouyang, Z.; So, L. Impacts of sewage irrigation on heavy metal distribution and contamination in Beijing, China. *Environ. Int.* 2005, 31, 805–812. [CrossRef] [PubMed]

25. Hajihashemi, S.; Mbarki, S.; Skalicky, M.; Noedoost, F.; Raeisi, M.; Brestic, M. Effect of wastewater irrigation on photosynthesis, growth, and anatomical features of two wheat cultivars (*Triticum aestivum* L.). *Water 2020*, 12, 607. [CrossRef]

26. Rodríguez-Morgado, B.; Gómez, I.; Parrado, J.; García-Martínez, A.M.; Aragón, C.; Tejeda, M. Obtaining edaphic biostimulants/biofertilizers from different sewage sludges. Effects on soil biological properties. *Environ. Technol.* 2015, 36, 2217–2226. [CrossRef] [PubMed]

27. Olanrewaju, O.S.; Glick, B.R.; Babalola, O.O. Mechanisms of action of plant growth promoting bacteria. *World J. Microbiol. Biotechnol.* 2017, 33, 197. [CrossRef]

28. Jastrzębska, M.; Kostrzewska, M.K. Using an environment-friendly fertilizer from sewage sludge ash with the addition of bacillus megaterium. *Minerals* 2019, 9, 423. [CrossRef]

29. Xu, L.; Geelen, D. Developing biostimulants from agro-food and industrial by-products. *Front. Plant Sci.* 2018, 9, 1567. [CrossRef]

30. Rodríguez-Morgado, B.; Caballero, P.; Paneque, P.; Gómez, I.; Parrado, J.; Tejeda, M. Obtaining edaphic biostimulants/biofertilizers from sewage sludge using fermentative processes. Short-time effects on soil biochemical properties. *Environ. Technol.* 2019, 40, 399–406. [CrossRef]

31. Shchegolkova, N.M.; Krasnov, G.S.; Belova, A.A.; Dmitriev, A.A.; Kharitonov, S.L.; Klimina, K.M.; Melnikova, N.V.; Kudryavtseva, A.V. Microbial community structure of activated sludge in treatment plants with different wastewater compositions. *Front. Microbiol.* 2016, 7, 90. [CrossRef]

32. Lane, D.J. 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, 1st ed.; Stackebrandt, E., Goodfellow, M., Eds.; John Wiley & Sons, Inc.: New York, NY, USA, 1991; pp. 115–175.

33. Przemieniecki, S.W.; Kurowski, T.P.; Damaszek, M.; Krawczyk, K.; Pszczółkowska, A.; Karwowska, A.; Mieczkowska, I.; Parrado, J.; Tejada, M. Obtaining of *edaphic* *biofertilizers* from different sewage sludges. *J. Agric. Sci. Tech.* 2019, 20, 609–619. [CrossRef]

34. Liu, X.; Liu, C. Effects of drought-stress on fusarium crown rot development in barley. *PLoS ONE* 2016, 11, e0167304. [CrossRef] [PubMed]

35. Przemieniecki, S.W.; Kurowski, T.P.; Potlaw, K.; Krawczyk, K.; Damaszek, M.; Karwowska, A. Plant growth promoting properties of *Serratia fonticola* ART 8 and *Pseudomonas putida* ART 9 and their effect on the growth of spring wheat (*Triticum aestivum* L.). *Environ. Biotechnol.* 2016, 35, 35–39. [CrossRef]

36. Mahmood, A.; Turgay, O.C.; Farooq, M.; Hayat, R. Seed biopriming with plant growth promoting rhizobacteria: A review. *FEMS Microbiol. Ecol.* 2016, 92, 112. [CrossRef]

37. Ahemad, M.; Khan, M.S. Effect of fungicides on plant growth promoting activities of phosphate solubilizing *Pseudomonas putida* isolated from mustard (*Brassica compestris*) rhizosphere. *Chemosphere* 2012, 86, 945–950. [CrossRef] [PubMed]

38. Przemieniecki, S.W.; Kurowski, T.P.; Kotlarz, K.; Krawczyk, K.; Damaszek, M.; Pszczółkowska, A.; Kacprzak-Siuda, K.; Chareńska, A.; Mastalerz, J. Bacteria isolated from treated wastewater for biofertilization and crop protection against fusarium spp. pathogens. *J. Soil Sci. Plant Nutr.* 2019, 19, 11. [CrossRef]

39. Przemieniecki, S.W.; Kurowski, T.P.; Karwowska, A. Plant growth promoting potential of *Pseudomonas* sp. SP0113 isolated from potable water a closed water well. *Arch. Biol. Sci.* 2015, 67, 663–673. [CrossRef]

40. Hudc, K.; Muchová, D. Influence of temperature and species origin on Fusarium spp. and *Microdochium nivale* pathogenicity to wheat seedlings. *Plant Protect. Sci.* 2010, 46, 59–65. [CrossRef]
41. Abuamsha, R.; Salman, M.; Ehlers, R. Effect of seed priming with Serratia plymuthica and Pseudomonas chlororaphis to control Leptosphaeria maculans in different oilseed rape cultivars. *Eur. J. Plant Pathol.* **2011**, *130*, 287–295. [CrossRef]

42. Goswami, D.; Vaghela, H.; Parmar, S.; Dhandhukia, P.; Thakker, J. Plant growth promoting potentials of *Pseudomonas* spp. Strain OG isolated from marine water. *J. Plant Interact.* **2013**, *8*, 281–290. [CrossRef]

43. Cummings, S.P. The application of plant growth promoting rhizobacteria (PGPR) in low input and organic cultivation of graminaceous crops; potential and problems. *Environ. Biotechnol.* **2009**, *5*, 43–50.

44. Witte, C.P. Urea metabolism in plants. *Plant Sci.* **2011**, *180*, 431–438. [CrossRef] [PubMed]

45. Przemieniecki, S.W.; Kurowski, T.P.; Damszel, M.; Karwowska, A.; Adamiak, E. Effect of Roundup 360 SL on survival of *Pseudomonas* sp. SP0113 strain and effective control of phytopathogens. *J. Agric. Sci. Tech.* **2017**, *19*, 1417–1427.

46. Przemieniecki, S.W.; Kurowski, T.P.; Kotlarz, K.; Mastalerz, J. The ability of *Pseudomonas* sp. SP0113 to solubilize tricalcium phosphate and its influence on the development of spring wheat. *Pol. J. Environ. Stud.* **2019**, *28*, 3533–3538. [CrossRef]

47. Ben Rebah, F.; Prévost, D.; Yezza, A.; Tyagi, R.D. Agro-industrial waste materials and wastewater sludge for rhizobial inoculant production: A review. *Bioresour. Technol.* **2007**, *98*, 3535–3546. [CrossRef]

**Publisher’s Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).