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ARTYKUŁ ORYGINALNY

Endofity z liści tytoniu jako potencjalne stymulatory wzrostu i czynniki kontroli biologicznej u pszenicy ozimej

Tobacco leaf endophytes as potential growth promoters and biocontrol agents in winter wheat

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Streszczenie

Bakterie endofityczne odgrywają kluczową rolę w utrzymaniu zdrowia roślin działając jako bakterie promujące wzrost (PGPB) oraz czynniki biokontroli zwalczające patogeny roślinne. Celem pracy była ocena potencjału bakterii endofitycznych wyizolowanych z liści tytoniu (*Nicotiana tabacum* L.) do stymulowania wzrostu pszenicy ozimej (*Triticum aestivum* L.), a także do ograniczania rozwoju patogennych grzybów *Fusarium oxysporum* i *Fusarium solani*. Dwadzieścia izolatów bakteryjnych przebadano pod kątem właściwości biochemicznych, tolerancji na zasolenie oraz aktywności antagonistycznej wobec patogenów grzybowych. Cztery szczepy (*Glutamicibacter halophytocola*, *Bacillus mobilis*, *Microbacterium paraoxydans* i *Huaxiibacter chinensis*) uznano za najbardziej obiecujące ze względu na ich zdolność do promowania wzrostu roślin oraz hamowania rozwoju grzybów. Doświadczenia szklarniowe potwierdziły ich pozytywny wpływ na wzrost pszenicy, wykazując istotny statystycznie przyrost w porównaniu z grupą kontrolną: długości łodygi (do 22,3%), świeżej biomasy (do 65,3%) oraz suchej biomasy (do 44,0%). Należy jednak zaznaczyć, że choć aktywność przeciwgrzybowa była wyraźna w warunkach *in vitro*, nie przełożyła się na mierzalną redukcję objawów chorobowych w warunkach szklarniowych. Wyniki te podkreślają zarówno potencjał, jak i ograniczenia endofitów pochodzących z tytoniu. Badanie to dostarcza wstępnych informacji na temat możliwości ich wykorzystania w rolnictwie zrównoważonym jako bionawozów oraz biologicznych środków ochrony roślin. Dalsze prace badawcze powinny koncentrować się na wyjaśnieniu mechanizmów ich działania oraz ocenie skuteczności w warunkach polowych, co pozwoli na optymalizację strategii ich aplikacji.

Słowa kluczowe: bakterie endofityczne, PGPB, *Triticum aestivum*, biokontrola, grzyby patogeniczne

Abstract

Endophytic bacteria play a crucial role in plant health, acting as plant growth-promoting bacteria (PGPB) and biocontrol agents against plant pathogens. This study aimed to evaluate the potential of endophytic bacteria isolated from tobacco leaves (*Nicotiana tabacum* L.) to promote winter wheat (*Triticum aestivum* L.) growth and inhibit the growth of the pathogenic fungi *Fusarium oxysporum* and *Fusarium solani*. Twenty bacterial strains were screened for their biochemical traits, salt tolerance, and antagonistic activity against fungal pathogens. Four strains (*Glutamicibacter halophytocola*, *Bacillus mobilis*, *Microbacterium paraoxydans*, and *Huaxiibacter chinensis*) were identified as the most promising due to their ability to enhance plant growth and inhibit fungal growth. Greenhouse trials confirmed their growth-promoting effects, with significant increases up to 22.3% in stem length, fresh biomass up to 65.3%, and dry biomass up to 44.0% compared to the control. However, while antifungal activity was evident *in vitro*, it did not result in a measurable reduction of disease symptoms under greenhouse conditions. These findings highlight the dual potential and limitations of tobacco-derived endophytes. This study provides preliminary insight into their possible use as biofertilizers and biocontrol agents in sustainable agriculture. Further research should focus on elucidating their mechanisms of action and testing their performance in field conditions to optimize application strategies.

Keywords: endophytic bacteria, PGPB, *Triticum aestivum*, biocontrol, pathogenic fungi

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Wstęp / Introduction

Potential of endophytic bacterial isolates as biostimulants in sustainable agriculture. In recent years, endophytic microorganisms have been highlighted as important and allies in sustainable agriculture (Semenzato and Fani 2024). The use of these microorganisms as exogenous inoculants is a promising strategy to enhance crop productivity. These bacteria and fungi colonize plant tissues without causing disease and often establish beneficial relationships that support plant development, improve resistance to pathogens, and help plants cope with environmental stress. These bacteria facilitate nutrient uptake, reduce the impacts of abiotic and biotic stresses, and increase plant immunity (Ali et al. 2024; Semenzato and Fani 2024). Specific strains isolated from various hosts have shown remarkable potential when applied to different crops. For instance, isolates belonging to the genera *Bacillus*, *Pseudomonas*, and *Azospirillum* are widely studied for their ability to recolonize internal tissues and trigger systemic resistance (Santoyo et al. 2016; Liu et al. 2022). Tobacco is known to harbour a rich and diverse endophytic microbiome across roots, stems and leaves, which varies with developmental stage and environmental conditions. Tobacco was selected as a source of endophytic bacteria due to its well-documented association with diverse microbial endophytes and its suitability as a model plant for microbiome studies (Yuan et al. 2018; Zhang et al. 2024). Endophytic bacteria, like those isolated from plants like tobacco, possess a range of properties that can enhance the health of plants and confer protection against pathogens. These bacteria are ecologically specialized to the niches of some plants so that they can better break through the plant's defence mechanisms than rhizosphere microorganisms described as plant growth- promoting rhizobacteria (PGPR). One of the main advantages of using endophytic bacteria that regain their endophytic nature once applied is their increased resistance to biotic and abiotic infections from the external plant environment (Lovecká et al. 2023). The need for such innovative solutions is driven by the significant impact of pathogens on global food security (Savary et al. 2019; Lovecká et al. 2023).

In agricultural production, economic losses caused by disease pathogens have a significant impact on the quantity and quality of plant crops obtained. Without crop protection, for instance, the yield loss of one of the world's most important cereals, wheat (*Triticum aestivum* L.), is approximately 50%. Even with plant protection methods mechanical, biological, or chemical, the potential loss is reduced to about 29%, with plant diseases accounting for around one-third of the total losses (Oerke 2006). Hoffmann et al. (2021) highlights the importance of finding sustainable and effective solutions to manage plant diseases and improve crop productivity.

PGPB – charakterystyka i mechanizmy działania / PGPB – characteristics and mechanisms of action

Plant growth-promoting bacteria (PGPB), particularly endophytic bacteria, play a crucial role in promoting plant growth and health through numerous mechanisms. The beneficial effects of PGPB are largely associated with their ability to synthetize phytohormones such as auxins, leading to enhanced root growth and more efficient nutrient absorption. Other than their capability of stimulating plant growth, endophytic bacteria also solubilize major nutrients such as phosphorus and nitrogen, which are important for plant growth. Beyond improving plant survival, these processes can also limit the use of chemical fertilizers, which supports the development of more sustainable agriculture (Santoyo et al. 2016; Negi et al. 2024).

Furthermore, endophytic PGPB strengthen plant defence systems by producing antimicrobial compounds that suppress phytopathogens. By inhibiting the growth of harmful fungi such as *Fusarium solani* and *Fusarium oxysporum*. These bacteria provide natural protection and contribute to the reduction in synthetic pesticide use. This integrated biocontrol capacity, combined with growth promotion, makes PGPB a valuable tool in the context of sustainable crop production (Aquino et al. 2019).

Problemy związane z grzybami chorobotwórczymi w uprawach rolniczych / Problems related to pathogenic fungi in agricultural crops

Even though there are numerous benefits linked with endophytic bacteria (Krawczyk et al. 2016; Setiawati et al. 2018; Simlat et al. 2023), the control of pathogenic fungi is a significant problem since, these pathogens cause enormous crop losses. They are particularly more problematic since they can infect various plant species and possess high genetic diversity, thus it is extremely difficult to control them through normal practices. Traditional chemical control is usually inadequate or ecologically harmful, thus the need for alternatives (McLaughlin et al. 2023).

The employment of endophytic microorganisms as biocontrol agents is an effective solution. These microbes, particularly those of the *Bacillus* and *Pseudomonas* species, have also shown effective antimicrobial action against certain fungal diseases. For example, microbes like *Pseudomonas fluorescens* and *Bacillus subtilis* produce antibiotics such as phenazine-1-carboxylic acid and subtilin, which suppress plant pathogens and promote plant health (Pertot et al. 2017; Toral et al. 2018). Additionally, endophytic bacteria can produce lytic enzymes and siderophores, further inhibiting pathogen growth and competing for essential resources like iron (especially ferric iron Fe^{3+}) (Sasirekha and Srividya 2016; Oukala et al. 2021). The biocontrol potential of endophytic bacteria, when applied to agricultural practices,

not only helps reduce reliance on chemical pesticides but also promotes more environmentally friendly and sustainable farming methods (Rakhalaru et al. 2025). However, despite their potential, the full application of endophytic bacteria as biocontrol agents requires further research to understand the dynamics of their interactions within different plant systems and optimize their use in crop protection (Sen et al. 2026). The effective control of *Fusarium*-induced plant diseases is crucial to minimize crop losses and decrease mycotoxin production in food item. Since mycotoxin production may occur during both pre-harvest and post-harvest stages, preventing their formation in the plant represents an important strategy to reduce contamination of food and feed (Todorović et al. 2023).

The aim of this study was to evaluate the potential of endophytic bacteria isolated from tobacco leaves as plant growth-promoting bacteria (PGPB) and their biocontrol capabilities against the pathogenic fungi *F. oxysporum* and *F. solani*. The research focused on identifying the mechanisms of action of these microorganisms, assessing their impact on the biomass accumulation of winter wheat (*Triticum aestivum* L.), including shoot length and fresh weight, and determining their effectiveness in suppressing fungal pathogens. The findings may contribute to the development of sustainable and eco-friendly agricultural practices by harnessing the natural interactions between beneficial microorganisms and plants.

Materiały i metody / Materials and methods

Izolacja bakterii / Bacteria isolation

Endophytic bacteria were isolated from healthy leaves of tobacco (*Nicotiana tabacum* L., var. *Xanthi*, grown under greenhouse conditions at the Department of Virology and Bacteriology Institute of Plant Protection – National Research Institute in Poznań. Plants were harvested at the stage of three leaves. First, fresh, healthy tobacco leaves are collected and thoroughly washed under running water to remove surface contaminants. The leaves are then surface sterilized by immersion in 70% ethanol for 30 s, followed by a 2% sodium hypochlorite solution for 2 min, and subsequently rinsed several times with sterile distilled water for 30 s. The sterilized leaves are cut into small fragments, including the central vein, which are then homogenized in a sterile physiological saline solution (0.85% NaCl) using a sterile mortar and pestle. The homogenate was subjected to serial ten-fold dilutions (10^{-1} –10^{-3}) and 100 µl of each dilution was inoculated in triplicate onto tryptic soy agar (TSA, Sigma-Aldrich, St. Louis, MO, USA) plates and incubated in the temperature of 28°C for 4 days. After incubation, morphologically distinct bacterial colonies were selected based on differences in colony size, shape and colour. The selected colonies were purified by repeated streaking on TSA plates and subsequently subjected to biochemical tests to evaluate their plant growth-promoting properties.

Właściwości wspomagające wzrost roślin / Plant growth-promoting properties

Selected bacterial isolates were screened for plant growth-promoting traits, including hydrogen cyanide (HCN) production, siderophore production, cellulase activity, auxin (IAA) production, motility, phosphate solubilization, lipase activity, salt tolerance, and ammonia production.

Hydrogen cyanide (HCN) production was determined using the picrate filter paper method. The bacterial isolates were grown on nutrient agar supplemented with glycine. A filter paper soaked in a solution of picric acid and sodium carbonate was placed inside the lid of each Petri dish. The plates were sealed and incubated at 28°C for 48–72 h. A color change of the filter paper from yellow to orange or reddish-brown was considered indicative of HCN production (Ghodsalavi et al. 2013).

Cellulase production was evaluated by growing bacteria on TSA followed by flooding the medium with a hexadecyltrimethylammonium bromide solution to precipitate undegraded carboxymethyl cellulose (CMC). Clear zones around the colonies indicated cellulase activity. The size of the transparent zone was measured, and a ratio of the zone's size to the colony diameter was calculated to assess enzyme activity (Hankin and Anagnostakis 1977).

Auxin production, an indicator of plant growth-promoting potential, was determined using biochemical assays. Bacterial strains were incubated in nutrient broth containing tryptophan, followed by centrifugation. The supernatant was mixed with Salkowski reagent, and the appearance of a red colour indicated auxin production. The intensity of the red colour was quantified by measuring absorbance at 535 nm (Ghodsalavi et al. 2013).

Motility was assessed by inoculating bacteria into semi-solid motility agar and observing the spread of growth after 24 and 48 hours of incubation at 27°C. A diffuse zone of growth around the inoculation point indicated motility, which is important for colonizing the rhizosphere, interacting with plants, and accessing nutrients, thereby supporting plant health (Schaad et al. 2001).

Phosphate solubilization was measured on Pikovskaya's agar medium containing insoluble phosphate sources. After 7 days of incubation, clear zones around the colonies indicated phosphate solubilization, demonstrating the bacterial ability to release available phosphate from the medium and enhance plant nutrient uptake (Sharma et al. 2011). Lipase production was evaluated by growing bacterial cultures on a medium containing Tween 20. Lipase activity was indicated by the formation of a precipitates around the colonies. Activity was scored from 0 (no activity) to 4 (very high activity) based on the size of the deposit (Ghodsalavi et al. 2013).

Siderophore production was tested using succinate medium, with fluorescence under UV 365 nm light after 48 hours confirming the presence of pyoverdines, specific to *Pseudomonas* spp., a type of fluorescent siderophore.

Salt tolerance was determined by inoculating bacterial strains into a 1% proteose peptone solution with varying sodium chloride concentrations (0%, 2%, 5%, and 10 the cultures were incubated at 28°C for 24 h under shaking conditions (120 rpm). Bacterial growth was assessed by measuring optical density at 600 nm (OD 600). The applied NaCl concentrations were selected to evaluate bacterial tolerance to moderate and high salinity stress.

Ammonia production was tested by incubating bacterial strains in proteose peptone broth. After 24 hours, Nesseler's reagent was added, and the development of a yellow colour indicated ammonia production. The intensity of the yellow coloration correlated with the amount of ammonia produced (Ghodsalavi et al. 2013).

Biokontrola / Biocontrol

The dual culture test was conducted to evaluate the ability of bacteria to inhibit the growth of pathogenic fungi. The pathogenic fungal strains used in this study were obtained from Bank of Pathogens of the Institute of Plant Protection – National Research Institute in Poznań initially, the fungi *F. oxysporum* and *F. solani* were inoculated on potato dextrose agar (PDA, Sigma-Aldrich, St. Louis, MO, USA) medium and incubated for 10 days at 25°C to allow full mycelial development. Subsequently, 5 mm mycelial plugs were cut using a sterile cork-borer and placed on fresh PDA medium. At a distance of 4 cm from the fungal plug, 10 µl of bacterial suspension at a concentration of 10^8 CFU/ml was applied. The plates were incubated at 25°C for an additional 5–7 days, depending on the incubation requirements of the specific fungi. After incubation, the inhibition zone around the bacterial colonies was assessed. The size of the inhibition zone was measured in millimetres (Zhang et al. 2014).

The biocontrol efficacy was expressed as the percentage inhibition of fungal growth, calculated using the percentage inhibition of radial growth (PIRG) index (Go et al. 2023). PIRG was calculated according to the formula:

$$\mathrm{PIRG} = \frac{R1 - R2}{R1} \times 100\%$$

R1 – represents the radius of fungal growth on the control plate,

R2 – represents the radius of fungal growth on the test plate with bacteria.

The experiment was performed in triplicate to ensure reproducibility of the results.

Eksperyment szklarniowy do oceny biokontroli / Greenhouse experiment for biocontrol evaluation

The greenhouse experiment was conducted at 17–21°C, with high substrate moisture maintained throughout the experimental period. Disease symptoms were assessed three weeks after fungal inoculation based on lesion development, discoloration, necrosis, and plant survival. To assess the biocontrol potential of the selected bacterial strains under greenhouse conditions, an experiment was conducted using winter wheat plants. The plants were first treated with bacterial suspensions containing known inoculum concentrations of 1.5×10^8 of the bacterial strains that exhibited positive biocontrol activity in dual culture assays. After 3 days, the same plants were inoculated with fungal suspensions of the pathogens that showed positive results in the dual culture tests. A conidial suspension was prepared at a concentration of approximately 1×10^6 conidia/ml for fungal pathogens, supplemented with 0.01% Tween-20 to ensure uniform distribution. Inoculation was performed by spraying the plants until runoff, while simultaneously applying the inoculum to the soil at the base of the stem. Following inoculation, plants were maintained under high humidity conditions for 24 h to promote infection. This combined treatment constituted the experimental group. Control plants were treated with only the fungal suspension, without prior bacterial inoculation. The plants were monitored for disease symptoms over a 14 days. The purpose of this greenhouse trial was to evaluate whether the bacterial strains could provide effective biocontrol of fungal pathogens in a controlled environment, like the results observed in *in vitro* assays. Disease symptoms, including lesion size, discoloration or necrosis, and plant mortality, were recorded to assess the efficacy of bacterial treatment in reducing fungal infection.

Fungal isolates were cultured on PDA (potato dextrose agar, Difco) for 10–14 days at 23–25°C. To prepare the inoculum suspension, cultured plates were scraped with approximately 5 ml of autoclaved 0.01% Tween-20 solution to reduce surface tension and facilitate spore liberation and mycelium removal. The suspension was then filtered through at least 2 layers of autoclaved cheesecloth. The filtrate was collected, and the mycelium was washed. The filtrate was transferred into a 20 ml tube and centrifuged at 4,000 × g for 8 min at room temperature. After carefully discarding the liquid supernatant, the conidia were resuspended in sterilized water. The number of conidia was counted using a hemacytometer, and the conidial concentration was adjusted to 10^7 spores/ml for *F. oxysporum* and *F. solani*.

The spore suspensions were injected into the seedling stems using a 10 ml syringe with a needle (0.7 mm outside diameter). The injection site was located at the one-leaf stage, approximately 0.5 cm above the soil surface. The injection was continued until the suspension flowed out from the top of the seedlings. Control plants were inoculated with the respective pathogen strain. The inoculated plants were covered with moistened plastic bags to maintain a saturated atmosphere facilitating infection. The bags were removed 48 hours post-inoculation. The greenhouse experiment was conducted at a temperature of 17–21°C, maintaining approximately 60–80% of water holding capacity throughout the experiment. After 3 weeks, plant infection was assessed.

Identyfikacja bakterii / Bacteria identification

Bacterial strains were identified using a biochemical-physiological method with the Biolog Gen III MicroPlate™ system (Biolog Inc., Hayward, CA, USA; software v. 2.8.0) according to the manufacturer's instructions (Protocol A). Fresh bacterial cultures grown on tryptic soy agar were used to prepare cell suspensions in inoculating fluid A, which were adjusted to 90–95% transmittance using a Biolog turbidimeter. Subsequently, the standardized suspensions were inoculated into Gen III MicroPlates™ and incubated at 27°C for 24 h under aerobic conditions. The metabolic profiles were recorded using a Biolog MicroStation™ system and analyzed with the Biolog database. Molecular identification was additionally performed by sequencing a fragment of the 16S rRNA gene. Polymerase chain reaction (PCR) products were sequenced commercially by Genomed S.A. (Warsaw, Poland) using the Sanger sequencing method. Bacterial genomic DNA was extracted using a modified CTAB protocol (Coleman Lab 2021). The extracted bacterial DNA was used as a template for amplification of the 16S rDNA gene fragment by PCR. The primer pair 16SA1 (5'-AGAGTTTGATCMTGGCTCAG-3') and 16SB1 (5'-TACGGYTACCTTGTTACGACTT-3') was applied, generating a product of approximately 1500 bp (Kikuchi et al. 2002). The PCR reaction was performed as follows. Initial denaturation was carried out at 94°C for 2 min. The next 35 reaction cycles had the following parameters: denaturation at 94°C for 45 s, primer attachment at 57°C for 45 s, and product elongation at 72°C for 1 min 30 s. Final elongation was carried out at 72°C for 9 min. The resulting PCR products were visualized by UV illumination after electrophoretic separation using the fluorescent dye Midori Green (Nippon Genetics Europe GmbH). Electrophoresis of the obtained DNA fragments was carried out in a 1% agarose gel with Midori Green. Into a single pocket in the gel, 3 µl of PCR product was applied with 1 µl of weights added. Separation was carried out in TBE buffer (0,5 X), at 75 V, for 40 min. PCR products were visualized under UV light. This step was designed to demonstrate the positive effect of the PCR reaction run and to determine the size of the resulting PCR product relative to the standard, molecular weight marker ØX174 (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The PCR reaction products were purified using the Qiaex II Gel Extraction Kit (Qiagen), according to the manufacturer's instruction. Chromas (www.basic.nwu.edu/biotools/Chromas.html) and FinchTV (www.geospiza.com/finchtv) software were used to analyze the quality and integrity of the obtained nucleotide sequences. The nucleotide sequences obtained from the single reads for each primer were then aligned and edited using BioEdit (Hall 1999). The consensus sequence of the 16S rRNA gene for each of the isolates analyzed was obtained using the GenDoc program (Nicholas et al. 1997). The consensus sequences obtained were compared, using the BlastN tool (www.ncbi.nlm.nih.gov/blastn), to all 16S rRNA sequences held in the Gene Bank resources (https://www.ncbi.nlm.nih.gov). The verified 16S rRNA sequences of the tested isolates were uploaded to the GenBank database for cataloguing and accession numbering.

Molekularna identyfikacja grzybów / Molecular identification of fungi

All fungal isolates were obtained from the pathogen collection of the Institute of Plant Protection – National Research Institute in Poznań. The isolates were cultured on PDA medium and incubated for 10–14 days at approximately 25°C. Genomic DNA was extracted from approximately 100 mg of fungal mycelium using a plant/fungal DNA isolation kit (Norgen Biotek, Canada) according to the manufacturer's instructions. Fungal material was ground into a fine powder in liquid nitrogen. At the end of the extraction, total genomic DNA was eluted in Elution Buffer B and stored at –20°C for longer term. The genetic diversity of the fungal isolates was evaluated based on sequences of the internal transcribed spacers (ITS) gene region. The ITS region was amplified using the universal fungal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al. 1990). PCR amplifications were performed in 20 µl PCR reactions volumes containing [1x PCR buffer, 1.5 mM $MgCl_2$, 200 µM of each dNTP, 0.5 µM of each primer, 1 U Taq DNA polymerase (Thermo Scientific), and approximately 50 ng of template DNA]. The amplification protocol consisted of an initial denaturation for 2 min at 95°C, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1 min, and extension at 72°C for 2 min, with a final extension at 72°C for 7 min. PCR amplification was performed in a thermal cycler (Biometra). The resulting PCR products were ~600–650 bp in length and were separated by electrophoresis on 1% agarose gels in 1x SB buffer, stained with Midori Green Advance DNA Stain (Nippon Genetics Europe GmbH), and visualized under UV light. Four microliters of each PCR reaction were loaded onto 1% agarose gels with MidoriGreen (Thermo Scientific); final concentration according to the manufacturer's instructions, along with appropriate molecular markers [GeneRuler 100 bp DNA Ladder, (Thermo Scientific)], and run in 1x SB buffer at 70 V for at least 40 minutes until the analyzed PCR fragments were clearly resolved. Gels were visualized and photographed under UV light. The remainder of each PCR reaction was utilized for sequencing. The consensus sequences obtained were compared, using the BlastN tool (www.ncbi.nlm.nih.gov/blastn), to all ITS sequences held in the Gene Bank resources (https://www.ncbi.nlm.nih.gov). The verified ITS sequences of the tested isolates were uploaded to the GenBank database for cataloguing and accession numbering.

Wyniki i dyskusja / Results and discussion

Izolacja bakterii i właściwości wspomagające wzrost roślin / Bacteria isolation and plant growth promoting properties

A total of 20 endophytic bacterial strains were isolated from tobacco leaves (*Nicotiana tabacum* L.). The results of performed biochemical assays, measuring the metabolite production representing bacterial growth under different conditions, are presented in (tab. 1). Based on the obtained results, four strains (G.HAL, B.MOB, M.PAR, and H.CHI) were selected as the most promising candidates for further study. These strains exhibited diverse biochemical traits that may contribute to their potential as plant growth promoters, particularly in environments with varying salinity levels.

Based on the conducted studies, various strains of endophytic bacteria isolated from tobacco leaves exhibited diverse biochemical abilities that may influence their potential as plant growth-promoting bacteria (PGPB) and their biocontrol capabilities. Strain G.HAL demonstrated cellulase production and motility but did not show auxin production, phosphate solubilization, fluorescent siderophore production, ammonia production, lipase production, or hydrogen cyanide production. Strain B.MOB demonstrated cellulase production and lipase production at level 2 but did not show any other trait. Strain H.CHI demonstrated cellulase production, ammonia production and lipase production at level 1 but did not show auxin production, motility, phosphate solubilization, fluorescent siderophore production, or hydrogen cyanide production. Strain M.PAR demonstrated cellulase production, motility, and lipase production at level 4, along with the presence of ammonia production, but did not show auxin production, phosphate solubilization, fluorescent siderophore production, or hydrogen cyanide production. In addition to their biochemical properties, the tested strains exhibited varying degrees of salt tolerance, Strain G.HAL demonstrated moderate tolerance to NaCl, with OD600 values decreasing significantly at 2% NaCl but showing partial recovery at 5% and 10% NaCl. Strain B.MOB exhibited a continuous decline in growth with increasing salt concentrations. Strain M.PAR showed a similar pattern, with a pronounced decrease in OD600 at

Tabela 1. Wyniki testów biochemicznych przeprowadzonych na 6 szczepach bakteryjnych. Oceniono zdolność do produkcji celulazy, auksyn, amoniaku, lipazy oraz cyjanowodoru, a także ruchliwość, rozpuszczanie fosforanów, wytwarzanie sideroforów fluorescencyjnych oraz tolerancję na różne stężenia NaCl (0%, 2%, 5% i 10%). Produkcję metabolitów oceniano w skali od 0 do 4, gdzie 0 – oznacza brak produkcji, a 4 – bardzo wysoką produkcję. Dodatkowo podano wartości OD600 reprezentujące gęstość optyczną hodowli bakteryjnych prowadzonych w określonych warunkach

Table 1. Results of biochemical assays conducted on six bacterial strains. The assays assessed the production of cellulase, auxin, motility, phosphate solubilization, fluorescent siderophores, ammonia, lipase, hydrogen cyanide, and tolerance to various NaCl concentrations (0%, 2%, 5%, and 10%). The production of metabolites was scored on a scale from 0 to 4, where 0 – indicates no production and 4 – represents very high production. Additionally, the OD600 values are provided, representing the optical density of bacterial cultures grown under the specified conditions

Testowane właściwości biochemiczne – Tested biochemical trait												
Próbka Sample	ocena – score	tak/nie – yes/no							OD_{600}			
	szczep strain	produkcja celulazy cellulase production	produkcja auxin auxin production	ruchliwość motility	solubilizacja fosforanów phosphate solubilization	produkcja fluorescencyjnych sideroforów fluorescent siderophores production	produkcja cyjanowodoru hydrogen cyanide production	produkcja amoniaku ammonia production	produkcja lipazy lipase production	tolerancja 0% NaCl 0% NaCl tolerance	tolerancja 2% NaCl 2% NaCl tolerance	tolerancja 5% NaCl 5% NaCl tolerance	tolerancja 10% NaCl 10% NaCl tolerance
G.HAL	*Glutamicibacter halophytocola*	yes	no	yes	no	no	no	no	0	0.616	0.104	0.191	0.152
B.MOB	*Bacillus mobilis*	yes	no	no	no	no	no	no	2	0.489	0.125	0.076	0.059
M.PAR	*Microbacterium paraoxydans*	yes	no	yes	no	no	no	yes	4	0.587	0.134	0.125	0.022
H.CHI	*Huaxiibacter chinensis*	yes	no	no	no	no	no	yes	1	1.120	0.388	0.160	0.018

5% NaCl and almost no detectable growth at 10% NaCl, indicating high sensitivity to salt stress. Strain H.CHI displayed the highest OD600 in the absence of NaCl; however, its growth declined markedly at 2% NaCl and was minimal at 10% NaCl.

Biokontrola / Biocontrol

The study evaluated the efficacy of bacteria in inhibiting the growth of pathogenic fungi using the dual culture test. The average radius of fungal growth on control plates was 7 cm for *F. oxysporum* and 9 cm for *F. solani*. In the presence of bacterial treatment, the average radius decreased to 5 cm for *F. oxysporum* and 7 cm for *F. solani*, resulting in inhibition rates of 28.6% and 22.2%, respectively. Additionally, the bacterial strain G.HAL showed inhibitory activity against *F. solani*, reducing its radius from 9 cm to 7 cm, corresponding to 22.2% inhibition. For *F. oxysporum*, M.PAR and H.CHI strains decreased the fungal growth radius from 7 cm to 5.2 cm and 5.7 cm, corresponding to inhibition percentages of 25.7% and 18.6%, respectively. These results demonstrate a significant inhibitory effect of the tested bacterial strains on both fungal pathogens, with G.HAL and M.PAR showing particularly strong biocontrol potential (tab. 2).

Pomiary / Mesurements

The measurements included stem length, fresh weight, dry weight, and water content, with the latter expressed as a percentage. The results are summarized in (tab. 3) and (fig. 1–4) with all values compared to the control sample. While the bar chart (fig. 1) illustrates the average percentage changes compared to the control, the accompanying box plots (fig. 2–4) provide a detailed distribution and variability of the data for stem length, fresh weight, and dry weight, respectively.

The application of endophytic bacteria isolated from tobacco leaves had a noticeable impact on plant growth parameters. The average stem length in treatments inoculated with bacterial strains ranged from 40.0 cm (B.MOB) to 46.0 cm (M.PAR), compared to 37.6 cm in the control group, which is an increase in stem elongation by up to 22.3%. Fresh weight showed a substantial increase in bac-

Tabela 2. Zdolności do hamowania wzrostu grzybów patogenicznych przez badane szczepy bakterii (tak/nie). Stopień zahamowania wzrostu grzyba patogenicznego przez szczep bakterii. w odniesieniu do kontroli. podano w procentach (%)

Table 2. Antifungal activity of the tested bacterial strains against pathogenic fungi (yes/no). The inhibition rate of pathogenic fungal growth by the bacterial strains is expressed as a percentage (%) relative to the control

Zdolność szczepów bakteryjnych do biokontroli – Biocontrol abilities of bacterial strains				
Szczep bakteryjny/Grzyb patogeniczny Bacterial strain/ Pathogenic fungus	G.HAL	B.MOB	M.PAR	H.CHI
Fusarium oxysporum	yes (28.6%)	no	yes (25.7%)	yes (18.6%)
Fusarium solani	yes (22.2%)	no	no	no

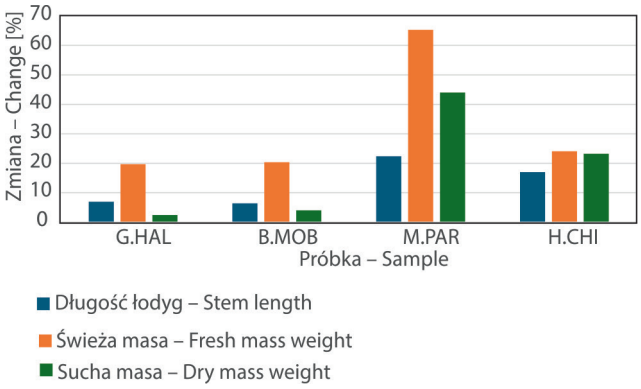
Tabela 3. Średnia długość źdźbła. świeża masa oraz sucha masa pszenicy ozimej traktowanej szczepami bakterii (G.HAL. B.MOB. M.PAR. H.CHI) w porównaniu do kontroli

Table 3. The average stem length. fresh weight and dry weight in winter wheat plants treated with bacterial strains (G.HAL. B.MOB. M.PAR. H.CHI) compared to the control

Próbka Sample	Średnia długość źdźbła Average stem length (±SD) [cm]	SEM	% zmiana długości źdźbła % change of stem length	Średnia świeża masa Mean fresh biomass (±SD) [g]	SEM	% zmiana świeżej masy % change of fresh biomass	p-value	Średnia sucha masa Mean dry biomass (±SD) [g]	SEM	% zmiana suchej masy % change of dry mass	p-value
G.HAL	40.2 ± 0.88	0.4415	(+6.91)	9.06 ± 0.20	0.104	(+19.66)	0.00015	1.28 ± 0.03	0.0158	(+2.4)	0.00015
B.MOB	40 ± 1.23	0.6177	(+6.38)	9.12 ± 0.18	0.0946	(+20.34)	0.00015	1.3 ± 0.02	0.0141	(+4)	0.00015
M.PAR	46 ± 0.65	0.3291	(+22.34)	12.51 ± 0.29	0.1471	(+65.30)	0.00015	1.8 ± 0.03	0.0163	(+44)	0.00015
H.CHI	44 ± 0.59	0.2972	(+17.02)	9.39 ± 0.17	0.0853	(+24.06)	0.00015	1.54 ± 0.03	0.0168	(+23.20)	0.00015
Kontrola Control	37.6 ± 0.65	0.3291	–	7.57 ± 0.17	0.0853	–	–	1.25 ± 0.02	0.0122	–	–

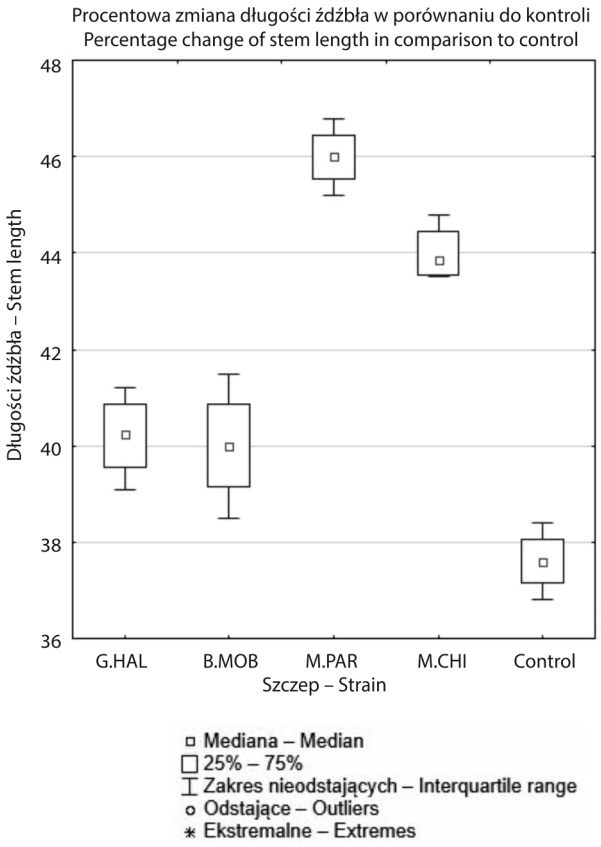
p-value – przyjęty poziom istotności wynosił 0.05. Jeżeli wartość p-value jest mniejsza od założonego poziomu istotności. hipoteza zerowa zostaje odrzucona. co pozwala stwierdzić występowanie istotnych statystycznie różnic między badanymi grupami

p-value – the significance level was 0.05. If the p-value is less than the predetermined significance level. the null hypothesis is rejected and it is concluded there are statistically significant differences between the groups



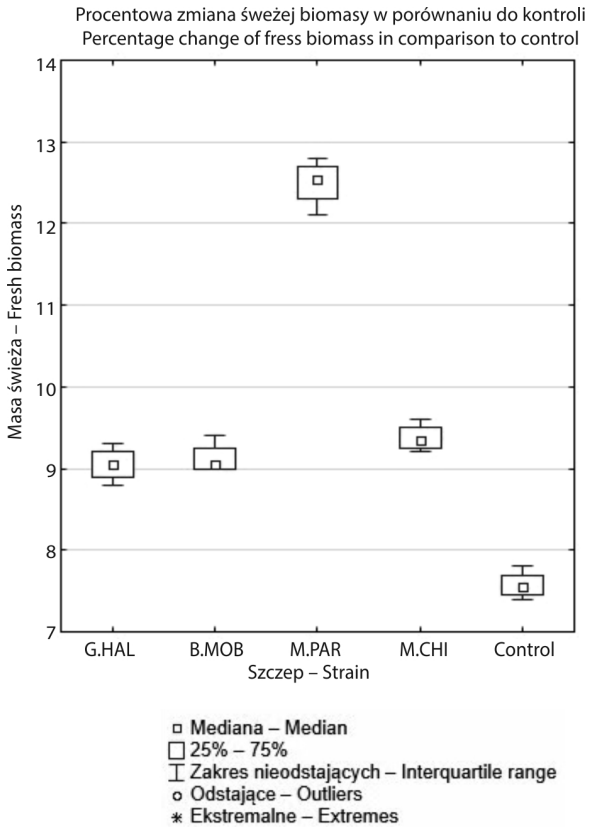
G.HAL – *Glutamicibacter halophytocola*, B.MOB – *Bacillus mobilis*, M.PAR – *Microbacterium paraoxydans*, H.CHI – *Huaxiibacter chinensis*

Rys. 1. Parametry wzrostu pszenicy ozimej wyrażone jako procentowa zmiana w stosunku do grupy kontrolnej
Fig. 1. Winter wheat growth parameters presented as a percentage change relative to the control group

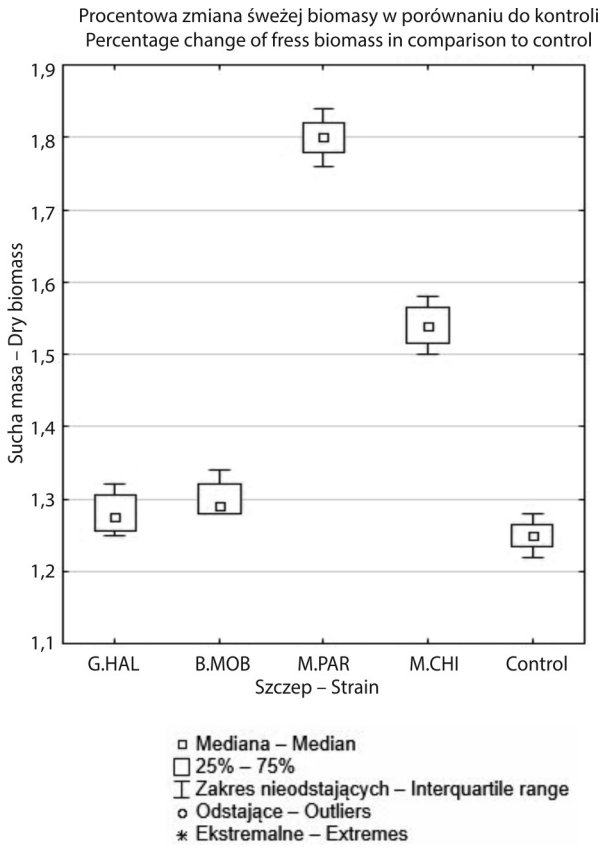


Rys. 2. Procentowa zmiana długości źdźbła w porównaniu do kontroli
Fig. 2. Percentage change of stem length in comparison to control

terial treatments, varying from 9.06 g (G.HAL) to 12.51 g (M.PAR), relative to 7.57 g in the control, representing up to a 65.3% increase. Similarly, dry weight ranged from 1.28 g (G.HAL) to 1.80 g (M.PAR), surpassing the control dry weight of 1.25 g by as much as 44%.



Rys. 3. Procentowa zmiana świeżej masy w porównaniu do kontroli
Fig. 3. Percentage change of fresh biomass in comparison to control



Rys. 4. Procentowa zmiana suchej masy w porównaniu do kontroli
Fig. 4. Percentage change of dry biomass in comparison to control

Wyniki eksperymentu szklarniowego / Greenhouse experiment results

The biocontrol potential of selected bacterial strains against fungal pathogens was evaluated under greenhouse conditions on winter wheat plants. Despite inoculation with bacteria at a high concentration (1.5×10^8 cells/ml) followed by fungal pathogen inoculation, no significant reduction in disease symptom development was observed in the experimental groups compared to the control plants inoculated with the pathogens alone.

Disease symptoms, including lesion size, discoloration, and plant mortality, developed with similar severity in both bacteria-treated plants and control groups. In particular, no significant reduction in lesion area or plant mortality or decrease in plant mortality was detected in the presence of bacterial treatment. These results indicate an absence of effective biocontrol of fungal pathogens by the tested bacterial strains *in vivo*, despite positive effects observed previously in *in vitro* assays.

Identyfikacja / Identification

All bacterial strains were identified using two methods: the Biolog Gen III system and 16S rRNA gene sequencing. The identification results are summarized in (tab. 4). The obtained sequences have been deposited in GenBank, and the corresponding accession numbers are provided.

All fungal strains were identified with ITS region sequencing. The identification results are summarized in (tab. 5). The obtained sequences have been deposited in GenBank, and the corresponding accession numbers are provided.

Endophytic bacteria are increasingly recognized as promising tools in sustainable agriculture. Their ability to promote plant growth and suppress phytopathogens positions them as valuable alternatives to chemical pesticides (Lovecká et al. 2023). In this study, four endophytic bacterial strains isolated from tobacco leaves (G.HAL, B.MOB, M.PAR, and H.CHI) demonstrated various plant-beneficial traits, including enzyme production, moderate salt tolerance, and majority of them showed antifungal activity against *F. oxysporum* and *F. solani*.

While suppressive soils are known for their complex microbiome that limits *Fusarium* disease development, key bacterial taxa commonly contributing to this effect include genera such as *Bacillus* and *Pseudomonas* (Todorović et al. 2023). However, the role of less-studied genera such as *Glutamicibacter* in *Fusarium* suppression remains large-

Tabela 4. Identyfikacja szczepów bakteryjnych na podstawie wyników uzyskanych z wykorzystaniem systemu Biolog Gen III (ver. 2.8) i sekwencjonowania regionu 16S rRNA oraz numery akcesyjne GenBank

Table 4. Identification of bacterial strains based on Biolog Gen III system (ver. 2.8) results, 16S rRNA gene sequencing, and GenBank accession numbers

Szczep Strain	Identyfikacja z użyciem Biolog Gen III Biolog Gen III identification	Identyfikacja przez sekwencjonowanie regionu 16S Region 16S sequencing identification	Stopień pokrycia Query cover [%]	Procent identyczności Percentage of identity [%]	Numer akcesyjny GenBank GenBank accesion number	Numer akcesyjny sekwencji o najwyższym stopniu podobieństwa GenBank accession number of the closest relative
H.CHI	no ID brak ID	*Huaxiibacter chinensis*	100	99	PX091148	NR_184601.1
M.PAR	*Microbacterium oxydans*	*Microbacterium paraoxydans*	100	100	PX091151	NR_025548.1
B.MOB	*Bacillus* sp.	*Bacillus mobilis*	99	100	PX091150	NR_184601.1
G.HAL	no ID brak ID	*Glutamicibacter halophytocola*	99	99	PX091152	NR_156872.1

Tabela 5. Molekularna identyfikacja izolatów grzybowych na podstawie wyników sekwencjonowania regionu ITS oraz numery akcesyjne GenBank

Table 5. Molecular identification of fungal isolates based on ITS region sequencing and GenBank accession numbers

Numer izolatu Isolate number	Identyfikacja przez sekwencjonowanie regionu ITS Region ITS sequencing identification	Stopień pokrycia Query cover [%]	Procent identyczności Percentage of identity [%]	Numer akcesyjny GenBank GenBank accesion number	Numer akcesyjny sekwencji o najwyższym stopniu podobieństwa GenBank accession number of the closest relative
2330	*Fusarium solani*	100	100	PX091155	NR_163531.1
2348	*Fusarium oxysporum*	100	100	PX091153	PX091153.1

ly unexplored. We observed that strain G.HAL showed the strongest plant growth promotion and antifungal potential. Identified as *Glutamicibacter halophytocola* produced cellulase and demonstrated motility. It inhibited fungal growth by 28.6% against *F. oxysporum*, and 22.2% *F. solani* as well as and improved wheat seedling parameters such as stem length and biomass. While *Microbacterium* species have been noted as components of suppressive soils, direct evidence for antifungal activity by *Microbacterium paraoxydans* has been limited. In contrast, our findings suggest that M.PAR may act through enzymatic degradation of fungal cell walls and competitive colonization, in line with mechanisms described by Tripathi et al. (2008) and Gao and Tao (2012). Recent studies demonstrate that bacteria from the family *Micrococcaceae*, to which *Glutamicibacter* belongs, may influence *Fusarium* populations by modulating the rhizosphere microbiome and producing enzymes that degrade fungal cell walls (Fu et al. 2021). This study provides preliminary evidence of its potential role in biological control, especially in environments with mild salinity. *Huaxiibacter chinensis* (H.CHI) also reduced fungal growth to varying extents. H.CHI, in particular, showed inhibitory activity against *F. oxysporum* with limited known literature on this genus.

Studies have shown that *Bacillus mobilis* exhibits ammonia production and cellulase activity, which may be associated with its potential antagonistic effects. Previous research, such as that by (Jiang et al. 2024) indicates that closely related *Bacillus* species effectively inhibit the growth of *F. oxysporum* and other plant pathogens. In contrast to previous reports (Jiang et al. 2024), our data show that *B. mobilis* did not suppress *Fusarium* growth, suggesting that its mode of action may differ from closely related *Bacillus* species. Despite the generally promising performance of these strains, none produced hydrogen cyanide or fluorescent siderophores, and only M.PAR showed consistent multi-enzyme activity. This suggests that the antifungal effects are likely related to enzymatic degradation and possibly competition for space and nutrients, rather than through siderophore-mediated iron chelation or volatile compound production. While some genera such as *Bacillus* and *Brevibacillus* are well established in literature for their production of antifungal metabolites (Todorović et al. 2023), the species identified in this study have received less attention. Specifically, no direct experimental confirmation exists in the literature for the antifungal capabilities of *G. halophytocola*, *H. chinensis*, or *B. mobilis* against *Fusarium* species. The presence of traits such as cellulase and lipase activity in these strains may explain their modest biocontrol potential, but the mechanisms remain unclear and require further testing under controlled and field conditions.

In addition to their antifungal properties, the bacterial strains tested in this study positively influenced the growth of winter wheat seedlings. Inoculation with strains such as M.PAR and G.HAL resulted in increased stem length and biomass compared to untreated controls. These growth-promoting effects may be attributed to the production of enzymes such as cellulases and lipases, as well as ammonia, which can improve nutrient availability and root development (Fialho de Oliveira et al. 2010). Although none of the strains produced auxin or solubilized phosphate, the observed improvements in plant growth suggest that other mechanisms, such as enhanced nutrient mobilization or modulation of the rhizosphere microbiome, may have contributed. These results support the idea that endophytic bacteria, even with limited classical PGP traits, can still promote plant development under certain environmental conditions.

In this study, the *G. halophytocola* strain G.HAL, isolated from tobacco leaves, exhibited a limited set of classical plant growth-promoting (PGP) traits. The strain produced cellulase and displayed motility but did not synthesize indole-3-acetic acid (IAA), solubilize phosphate, produce fluorescent siderophores, ammonia, lipase, or hydrogen cyanide. Nevertheless, G.HAL had a pronounced positive effect on wheat growth, increasing stem length by up to 22.3% and fresh weight by 19.9% compared to the control. The strain also demonstrated strong antagonistic activity against *Fusarium* sp., suggesting the involvement of biocontrol mechanisms independent of HCN or siderophore production. These results contrast with the findings of (Qin et al. 2018) who analyzed 15 *Glutamicibacter* strains, including *G. halophytocola* KLBMP 5180, for PGP characteristics. Most of the tested strains grew on nitrogen-free medium, produced 1-aminocyclopropane-1-carboxylate deaminase (ACCD), and a subset synthesized IAA and solubilized phosphate. Strain KLBMP 5180 promoted the growth of *Limonium sinense* both under saline and non-saline conditions, which the authors attributed to a combination of classical PGP mechanisms, including modulation of plant hormonal balance and mitigation of ethylene stress via ACCD activity. *Huaxiibacter chinensis*, originally isolated from clinical material, has not yet been described as a plant growth-promoting microorganism or as a biocontrol agent. Nevertheless, the presence of genes related to biofilm formation, motility, and colonization may suggest an endophytic potential, which requires further functional studies (Chen et al. 2025). In a recent study, (Khanna et al. 2022) evaluated endophytic bacteria from chickpea roots for growth promotion and antagonism against *Fusarium* wilt. Among twenty isolates, 35% produced siderophores, 15% produced HCN, and 55% grew well on ACC-supplemented medium. Based on 16S rRNA analysis, CRBE1, CRBE3, and CRBE7 were identified as *Priestia megaterium*, *Brucella haematophila*, and *Microbacterium paraoxydans*, respectively. CRBE3 and CRBE7 showed strong *in vitro* antagonism against *F. oxysporum* f. sp. *ciceris*, and seed inoculation led to significant biocontrol under screenhouse conditions.

In contrast, *G. halophytocola* (G.HAL) from tobacco leaves showed a limited set of classical PGP traits. It produced cellulase and was motile but did not synthesize IAA, solubilize phosphate, or produce siderophores, ammonia, lipase, or HCN. Nevertheless, G.HAL enhanced wheat growth, increasing stem length by 22.3% and fresh biomass by 19.9% compared with the control, and displayed clear *in vitro* antagonism against *Fusarium*, suggesting biocontrol mechanisms independent of HCN or siderophores. We observed that, unlike the chickpea endophytes described by Khanna et al. (2022), our tobacco leaf isolates did not significantly reduce disease severity under greenhouse conditions. Collectively, our findings highlight that endophytic isolates of tobacco leaves, and in particular *G. halophytocola* and *M. paraoxydans,* have significant aspects of plant growth promotion along with *Fusarium* antagonism, even in the absence of several conventional PGP markers. The biocontrol prospect of *H. chinensis*, a new report of a biocontrol bacterium, further illustrates the potential of underexplored taxa for plant protection in an eco-friendly method. But the *in vitro* seen antifungal activity was not consistently reflected in greenhouse pathogenesis suppression, and points toward the need for additional work with colonization efficiency, host-strain match, and indigenous microbiome compatibility. Future studies need to be directed toward clarifying the mechanism through genomic and metabolomic analysis and determining the biocontrol potential of the isolates through field trials.

Wnioski / Conclusions

1. Endophytic bacteria isolated from tobacco leaves show strong potential to promote plant growth, with strain M.PAR being the most effective and a promising candidate for biofertilizer development.
2. Although several strains exhibited clear antagonistic activity against fungal pathogens *in vitro*, this effect did not translate into significant disease reduction under greenhouse (*in vivo*) conditions.
3. The effectiveness of these bacteria as biocontrol agents depends on multiple factors, such as application method, timing, colonization efficiency, and microbiome interactions, indicating the need for further research under controlled and field conditions to fully harness their agricultural potential.
4. Tobacco leaf-derived endophytic bacteria represent a promising sustainable alternative to chemical fertilizers and pesticides, particularly for organic farming, although their practical application requires further validation.

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