Streszczenie w języku angielskim

One of the key challenges in using microbiological plant protection agents is their interactions with anthropogenic pollutants such as synthetic pesticide residues, MPs, and industrial dyes. Despite growing environmental awareness, regulatory changes, and increasing availability of biopesticides, synthetic pesticides still dominate in pest control. Many strains of bacteria from the *Bacillus* and *Pseudomonas* genera are notable for their ability to promote plant growth, inhibit the development of phytopathogenic fungi, and participate in soil bioremediation. In these processes, bacterial metabolites, including biosurfactants – compounds characterized by both surface activity and diverse biological activity – play a significant role.

The main objective of this study was to assess the antifungal activity of *Bacillus* and *Pseudomonas* strains capable of producing cLPs, also in the presence of selected anthropogenic pollutants.

The research begins with the characterization of biosurfactants produced by 80 rhizosphere bacterial strains (77 from genus *Bacillus* and three from genus *Pseudomonas*). The study included 62 *Bacillus* isolates capable of producing two or three cLP-Bc biosurfactants (surfactin, iturin, and/or fengycin). The initial pool of bacterial models also included strains that produce only one cLP-Bc or none at all. Three *Pseudomonas* isolates were also examined, and their produced biosurfactants were identified. It was found that the strains ASK 10, ASK 68, and Zg 9.3 produce, respectively, pholipeptin, a mixture of viscosin and HAA (a precursor of RLs), and milkisin C. As the research progressed, the number of strains used was gradually narrowed down.

In the next stage, the ability of bacteria to inhibit the growth of *Fusarium* fungi through non-volatile metabolites and VOCs was compared. Dual culture experiments on agar solid media showed that 22 out of 72 *Bacillus* isolates effectively colonized *F. sambucinum* IM 6525 hyphae. For these strains, intensive dendritic bacterial growth was observed, preventing the fungal mycelium from further colonizing the medium surface. These strains also demonstrated high iturin production (≥ 200 mg L^{-1}) and produced fengycin. However, no hyphal colonization was observed for the second *Fusarium* strain, *F. culmorum* DSM 1094.

Antifungal activity studies of 32 selected bacterial strains were then conducted in liquid media. It was found that most analyzed bacteria significantly inhibited the biomass growth of *Fusarium* fungi used in the study. The evaluation of bacterial VOCs production showed that all 32 strains emitted VOCs that inhibited the aerial mycelium growth of *F. culmorum* DSM 1094. In the case of several bacterial isolates, these metabolites also inhibited surface mycelium growth by no more than 30%, but had no effect on the mycelium of *F. sambucinum* IM 6525.

The next phase assessed the influence of bacteria on the growth of cucumber seedling roots. Cucumber seeds were incubated on MS medium and wet filter paper. Of the 32 strains tested, 53.1% inhibited cucumber root growth, 40.6% had no significant effect, and only 6.3% stimulated root development. The most beneficial effect was observed for *Bacillus* sp. Kol B9.

This section concluded with the genetic identification of 23 bacterial isolates. It was determined that most *Bacillus* strains belong to the *B. subtilis* species complex, with eight likely to be *B. subtilis*, five to be *B. amyloliquefaciens*, and five to be *B. velezensis*. Strain named Kol B3 may belong to either *B. amyloliquefaciens*, *B. velezensis*, or *B. methylotrophicus*. It was also found that strain named Kol Si4 most likely belongs to *B. subtilis* or *B. halotolerans*, while strain Zg 7.6 probably belongs to *B. pumilus*. The isolates ASK 10, ASK 68, and Zg 9.3 were identified as *P. koreensis*, *P. fluorescens*, and *Pseudomonas* sp., respectively.

In addition to biomass inhibition, cLP production, and antifungal VOCs production, other antifungal mechanisms against *Fusarium* were investigated. The impact of five bacterial strains (ASK 10, Kol B2, Kol B3, Kol B9, and Kol L6) on fungal membrane permeability, lipidome changes, and spore germination was assessed. It was found that high antifungal activity was associated with an increased hyphal membrane permeability. It was also shown that bacterial non-volatile metabolites (including biosurfactants) can variably affect *Fusarium* spores by inhibiting spore germination and hyphal growth or even causing spore lysis.

A significant portion of the study focused on the effects of various biotic and abiotic factors on biosurfactant production and antifungal activity of the tested strains. It was found that the presence of *F. culmorum* DSM 1094, *F. sambucinum* IM 6525,

or *F. oxysporum* KKP 458 fungi in bacterial-fungal co-cultures generally reduced surfactin and iturin levels.

It was also observed that in 10 out of 37 co-cultures containing two different *Bacillus* strains, the surface activity of the supernatants increased (suggesting higher biosurfactant production). However, subsequent increased antifungal activity was only noted in one cultivation, containing *F. oxysporum* KKP 458 mycelium and *Bacillus* sp. Kol D4 and *B. subtilis* DSM 3257 cells.

The study demonstrated that anthropogenic pollutants can differentially affect biosurfactant production and bacterial antifungal activity. This effect depended on the bacterial and fungal strain used, as well as the xenobiotic added to the culture.

It was found that azo dyes negatively affect the surface activity of post-culture broths, suggesting reduced biosurfactant production and/or activity. However, the presence of azo dyes in bacterial-fungal co-cultures did not negatively impact the antifungal activity of neither ASK 10 nor ASK 68 bacteria against *F. sambucinum* IM 6525. In fact, increased antifungal activity was observed when *P. fluorescens* ASK 68 and the RB5 or AMR azo dyes were both present in the cultivation.

It was also found that and addition of MP to *Bacillus* cultivation can stimulate bacterial growth, does not significantly affect surfactin production, but may impact iturin levels. It was determined that the synthetic herbicides 2,4-D and MET either reduced or did not change the antifungal activity of *Bacillus* strains against *Fusarium* fungi. In contrast, the synthetic fungicides AZ and PR either increased or had no effect on antifungal activity. An addition of synthetic pesticides to *Bacillus* cultivations either reduced, had no effect, or increased the levels of iturin and surfactin. This effect was dependent on the *Bacillus* strain, *Fusarium* strain, and the pesticide used.

The final stage of the study focused on the evaluation of the application potential of *Bacillus* strains with diverse antifungal mechanisms and no negative impact on cucumber seedling root growth. It was found that coating cucumber seeds with bacterial cells protected the seeds and seedlings from the harmful effects of *F. culmorum* DSM 1094 and *F. sambucinum* IM 6525, which spores were introduced into the soil. This effect was particularly pronounced when *Bacillus* sp. Kol B9 was used for the seed coating.

Despite the diverse effects of environmental pollutants on the biosurfactant production observed in this study, none of the applied compounds limited the antifungal activity of *Bacillus* sp. Kol B9, confirming its high application potential. However, there is a risk that the antifungal efficacy of these bacteria could be significantly reduced in the presence of multiple different pollutants. Such effect was observed for *Bacillus* sp. Kol L6 – although in many experimental setups the addition of synthetic herbicides or MP increased the antifungal activity of these bacteria, the simultaneous presence of all three pollutants led to a significant reduction of this effect.

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data i miejsce

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podpis