ENDOPHYTIC BACTERIA IN THE PHYTODEGRADATION OF PERSISTENT ORGANIC POLLUTANTS

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Abstract: Organic pollutants are released into the environment as a result of various human activities. Traditional physical and chemical methods for the clean-up of contaminated soil and water are often costly and invasive. A good alternative to the above methods is bacteria-assisted phytodegradation. Recently, particular attention has been focused on endophytic bacteria equipped with appropriate metabolic pathways, increasing the efficiency of organic compound degradation, and promoting plant growth. Endophytic bacteria are known to degrade various classes of organic compounds such as polycyclic aromatic hydrocarbons (PAHs), volatile and monomeric organic compounds, explosives as well as pesticides. Additionally, endophytic bacteria can promote the growth and development of plants through a wide range of direct and indirect mechanisms, which also affect the effectiveness of phytoremediation processes.


1. Introduction

Organic pollutants released into the environment as a result of industrial and agricultural activity pose a serious threat to all living organisms [1]. Since traditional, physical and chemical methods for remediation of degraded environments are often costly and invasive, the methods of biological removal of this type of pollutants from the environment are garnering increasing attention. One of them is phytodegradation, based on the ability of plants to transform organic pollutants [5, 11, 75]. In recent years, studies on application of degradation potential of bacteria have been carried out to increase the efficiency of this method. Special attention of researchers is focused on endophytic bacteria. These bacteria can promote the growth and development of plants and, consequently, increase their biomass. They may also affect the bioavailability of organic pollutants in the soil. An important advantage resulting from the application of this group of microorganisms to support phytodegradation processes is also the fact that all toxic organic compounds collected by the plant undergo mineralization within its tissues [1, 20, 30].
2. Phytodegradation of organic pollutants

Plant metabolism of xenobiotics sourced from the environment includes general transformations of these compounds into more water-soluble forms and the process of their sequestration [5, 75]. Phototrophic plants do not use organic compounds as a source of carbon and energy, and their role is limited to transformation of these compounds into less toxic forms, in order to protect sensitive enzyme systems and organelles. In the process of detoxification of xenobiotics in plant cells, three phases are distinguished: activation, conjugation and sequestration compartmentalization. In the first phase of transformation, reactive polar functional groups are introduced into the lipophilic organic compounds. This leads to a reduction in their lipophilicity and increased solubility in the aqueous environment of the cell. The first phase reactions are usually catalyzed by enzymes such as cytochrome P450 and carboxyl esterases. The second phase includes reactions catalyzed by glutathione S-transferases (GST) and glucosyltransferases (GT), reactions of coupling the transformed compounds with endogenous molecules such as amino acids, sugars or glutathione to reduce their phytotoxicity. In the third phase, removal of inactive xenobiotics from the cytosol into apoplastic cell compartments or vacuoles occurs [72].

The effectiveness of the phytodegradation process can be limited by plant sensitivity to excessively high concentrations of xenobiotics which can cause phytotoxic effects, inhibit plant growth and development [10]. It can also be limited by the bioavailability of pollutants [55]. The disadvantage of this process is also the – in many cases unknown – pathway of the transformation of compounds absorbed in plant tissues, which may lead to the formation of metabolites with significantly higher toxicity than the parent substance [36].

3. Sources of endophytic bacteria enhancing phytodegradation

In contrast to plants, microorganisms, due to their comprehensive metabolic abilities, enable complete degradation of organic compounds, to CO$_2$ and H$_2$O [21]. In comparison with other organisms, they are characterized by a unique ability to adapt to new environmental conditions, including the treatment of compounds which are not products of their own metabolic transformations as an energy and carbon source [14]. Therefore, in recent years, a significant role in the treatment of degraded environments has been attributed to phytoremediation supported by microorganisms. A special role in this process is attributed to endophytic bacteria equipped with appropriate metabolic pathways, which increase the efficiency of organic compounds degradation, help plants to overcome the stress caused by pollution, and promote their growth [5].

Endophytic bacteria are defined mainly as bacteria colonizing internal plant tissues without causing disease symptoms or negative impact on their plant host [60]. In practice, the term defines microorganisms which can be isolated from surface-sterilised plant organs [18]. Many bacterial endophytes have been isolated from both dicotyledonous and monocotyledonous plants, from woody species such as oak and pear, to crop plants such as sugar beet and maize [60].

Population and diversity of endophytes present in plants is variable, mainly dependent on the plant genotype, type of tissue, plant development phase, amount of organic contaminants and physicochemical conditions of soil [28, 54]. A study by Phillips et al. [54] has proven that different plant species growing in hydrocarbon-contaminated soil maintain separate populations of endophytic microorganisms which may affect the ability of plants to promote the degradation of specific hydrocarbon groups.

The presence of endophytic bacteria capable of degrading organic pollutants has been demonstrated in many plant species characterised by high tolerance/resistance to contamination, such as the poplar (Populus sp.) or willow (Salix sp.) [10, 81]. Bacteria useful in phytodegradation processes have also been isolated from tissues of the common trefoil (Lotus corniculatus), Italian ryegrass (Lolium multiflorum), yellow lupine (Lupinus luteus), peas (Pisum sativum), wheat (Tricicum durum) and maize (Zea mays) [39]. From the pedunculate oak (Quercus robur) and common ash (Fraxinus excelsior), endophytes with the potential for trichloroethylene (TCE) degradation [1] have been obtained, while in the nodules of legumes such as broad bean (Vicia faba) and white lupine (Lupinus albus) endophytes capable of decomposing both aliphatic and aromatic hydrocarbons [81] have been found. Plants inhabiting wetlands [20] and salt marshes [45] have also proven to be a good source of endophytic bacteria capable of using organic compounds as a carbon source. The strains isolated from these plants belong mainly to the genus of Burkholderia, Enterobacter, Pseudomonas and Pantoea [37].

It is worth noting that bacterial endophytes capable of degrading pollutants colonise specific plant tissues [1]. First of all, they can be found in xylem and/or root cortex [5]. The highest abundance of this group of bacteria has been observed in the roots of most plants [12, 59].

4. Organic pollutants degraded by endophytic bacteria

Polycyclic aromatic hydrocarbons (PAH) constitute a group of toxic and persistent organic pollutants, which are widespread in the environment [53]. The
presence of this type of compounds may be the result of both natural processes, such as forest fires and volcanic eruptions, as well as industrial and agricultural human activities [3]. Many endophytic bacteria are able to grow in the presence of PAHs, and some can degrade these compounds, using them as a source of carbon and energy. An example may be the endophytic strains *Pseudomonas putida* PD1 and *Pseudomonas* sp. P6h degrading phenanthrene [28, 66] or isolated from the Canadian horseweed (*Conyza canadensis*) and red clover (*Trifolium pratense*). *Stenotrophomonas* sp. P1 and *Pseudomonas* sp. P3 capable of degrading naphthalene, phenanthrene, fluorene, pyrene and benzo (a)pyrene [86].

The presence of phenol and its derivatives in the environment is associated with the widespread use of these compounds, among others, for the production of dyes, paints, varnishes, detergents, herbicides and medicines. These compounds can also be formed as by-products of many industrial processes, such as: petroleum processing, hard coal coking and steel production. They may also arise from the biodegradation of aromatic polymers, such as lignin and tannins [40]. The ability to degrade phenols has been demonstrated, inter alia, in endophytic bacteria isolated from roots and stems of hemp (*Cannabis sativa*), growing in areas contaminated with hydrocarbons [22]. Among the isolated bacteria, the greatest potential for degrading this aromatic substrate was demonstrated by three isolates, identified as: *Pseudomonas* sp. (AIEB-4), *Alcaligenes* sp. (AIEB-6) and *Achromobacter* sp. (AIEB-7). All three strains showed the ability to grow in the presence of 1000 mg/L ph. During the 96-hour culture, *Achromobacter* sp. (AIEB-7) was able to degrade 81% of the introduced dose of phenol, while *Pseudomonas* sp. (AIEB-4) and *Alcaligenes* sp. (AIEB-6) utilised 72% and 69% of the initial dose of phenol, respectively.

Among all types of pollution, one of the main threats to the environment and human health are pesticides. Chlorpyrifos [O, O-Diethyl O- (3,5,6-trichloro-2-pyridinyl) phosphorothioate] (CP) is an insecticide widely used in agriculture. This compound accumulates in the environment, posing a threat to people and animals. It has been shown that CP can cause liver and kidney damage and negatively affect sperm activity. In addition, it is considered one of the factors responsible for the occurrence of breast cancer in women. The ability to degrade this insecticide has been demonstrated in the *Sphingomonas* sp. strain, HJY, isolated from Chinese chives (*Allium tuberosum* Rottl. ex Spreng) [11].

2,4-dichlorophenoxyacetic acid (2,4-D) is one of the most widespread herbicides [37]. 2,4-D salts are easily absorbed by the roots of plants and undergo translocation into meristematic tissues of roots and shoots, where the compound acts as a plant hormone, causing their uncontrolled growth. The mobility of 2,4-D in soil often leads to the pollution of surface and groundwater. Although the herbicide is biodegradable, it may persist in soil and water [16]. The use of 2,4-D on a large scale poses a threat to both human health and the environment [37]. The ability of endophytic bacteria to degrade 2,4-D has been demonstrated in an experiment carried out by Germaine et al. [16]. In these studies, the endophytic strain *P. putida* VM1450, derived from internal poplar tissues (*Populus deltoids*), was introduced into peas (*Pisum sativum*). The inoculated plants were treated with 2,4-D. It was found that strain VM1450 actively colonised the internal tissues of the plant, and the inoculated plants were characterised by a greater ability to remove 2,4-D from the soil and did not accumulate this herbicide in the tissues [16].

Production and processing of ammunition led to environmental pollution with compounds such as: 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) or octa-hydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX). These toxic and mutagenic compounds are characterised by high durability and resistance to chemical agents, biological oxidation and hydrolysis [52]. A study by Van Aken et al. [73] has demonstrated that the endophytic strain of *Methylobacterium* sp. BJ001, isolated from leaves and roots of poplars (*Populus deltoids x nigra* DN34), exhibits the capability of degrading TNT, RDX and HMX. After 55 days of BJ001 strain culturing in the presence of the above compounds, total degradation of 25 mg/L TNT, 20 mg/L RDX and 2.5 mg/L HMX was found.

Selected bacterial species isolated from roots, shoots and rhizosphere of sycamore maple (*Acer pseudoplatanus*) growing in the soil contaminated with TNT, were used by Thijs et al. [71] to form a bacterial consortium CAP9. The consortium combines the ability to efficiently transform TNT and promote plant growth. It has been proven that the inoculation of the bent grass (*Agrostis capillaris*) with this bacterial consortium stimulated the growth of plants growing in soil contaminated with TNT. For the first time, these studies demonstrated that the endophytic bacterium *Stenotrophomonas chelatiphagus* isolated from the leaves and *Variovorax ginsengisola* strain isolated from the sycamore maple leaf can transform TNT into hydroxy-lamine and amino dinitrotoluene.

Currently, many studies focus on the possibility of using endophytic methanotrophs in the bioremediation of greenhouse gases, in particular methane and carbon dioxide. These bacteria contribute to the efficient capture of methane produced in peat bogs, thus limiting its emission into the atmosphere [67]. Methanotrophic bacteria are capable of using methane as the sole source of carbon and energy. The key enzyme responsible for oxidation of methane is methane monooxygenase.
This enzyme can exist in two forms, as sMMO and pMMO. The sMMO form is present in the cytosol, whereas pMMO is associated with the cell membrane. Both forms of this enzyme have been shown to oxidise a number of aliphatic and aromatic compounds [64] and may be present in the cell simultaneously, but their activity is directly dependent on the concentration of copper ions (Cu^{2+}) in the biomass [68]. It has been demonstrated that endophyte methanotrophs, *Methylocella palustris* and *Methylcopasa acidophila*, colonizing tissues of the moss of the genus *Sphagnum*, oxidise methane to carbon dioxide, which is then used by plants in the process of photosynthesis. Methanotrophic endophytes colonizing *Sphagnum* spp. can reduce the emission of CH₄ and CO₂ from peat bogs by as much as 50% [67]. Methanotrophic endophytes can also be used to inoculate plants such as: reed (*Phragmites* sp.), reedmace (*Typha* sp.) and willow (*Salix* sp.), inhabiting an artificial wetland system. Moreover, they can also be introduced into arable crops and contribute to the promotion of their growth [67].

5. Genetic basis of xenobiotics degradation in endophytic bacteria

In the genomes of the majority of isolated endophytic bacteria capable of degrading hydrocarbons, the gene encoding the alkane monoxygenase (*alkB*) and the gene encoding cytochrome P450 hydroxylase (*CYT153*) have been found [2, 27, 49]. For example, in the studies of Kukla et al. [29] it was proven that the majority of endophytic bacteria obtained from the perennial ryegrass (*Lolium perenne* L.), a plant growing in an environment contaminated with petroleum hydrocarbons, possessed the *CYT153* gene. In the genome of one of the obtained strains, *Rhodococcus fascians* L11, the presence of the gene *alkH* encoding alkane hydroxylase and the gene *pah* encoding the alpha subunit of PAH hydroxylating dioxygenases was found. Endophytic strains of *Microbacterium* sp. and *Rhodococcus* sp. (L7, S12, S23, S25) had the *alkB* gene in their genome. In the genome, none of the isolates obtained showed the presence of the *c230* gene encoding catechol 2,3-dioxygenase, the key enzyme in the degradation pathway of monocyclic aromatic hydrocarbons. In turn, in studies carried out by Siciliano et al. [63] it was shown that in the soils contaminated with petroleum, two genes encoding enzymes responsible for hydrocarbon degradation, **alkB** gene, encoding alkane monoxygenase and **ndoB**, encoding naphthalene dioxygenase were more common in endophytic bacteria than in the microorganisms living in the rhizosphere. Among the endophytic strains, genes encoding enzymes associated with the decomposition of nitro compounds, such as, for example, nitrotoluene monoxygenase (*ntnM*) and 2-nitrotoluene reductase (*ntdAa*) were also common [63]. However, the studies conducted by Andria et al. [4] on the expression of the *alkB* gene encoding the alkane monoxygenase in two bacterial strains showed that the endophytic strain *Pseudomonas* sp. ITR153 isolated from the endosphere of Italian ryegrass (*Lolium multiflorum* L.) exhibited a much higher level of expression of the *alkB* gene than the strain *Rhodococcus* sp. ITRH43, obtained from the rhizosphere of this plant. Moreover, the expression of the *alkB* gene occurred not only in the rhizosphere but also *in planta*.

Differences in the expression of genes encoding degradation enzymes in endophytic bacteria were also demonstrated by Yousaf et al. [82] in the studies on the expression of the *CYP153* bacterial gene encoding cytochrome P450 hydroxylase, an enzyme involved in hydrocarbon degradation. In these studies, three different plant species: Italian ryegrass (*Lolium multiflorum* var. Taurus), the common Birdsfoot trefoil (*Lotus corniculatus* var. Leo) and alfalfa (*Medicago sativa* var. Harpe) were inoculated with three different strains of *Enterobacter ludwigii*. It was found that the bacterial strains introduced into plants differed significantly in the level of expression and the number of genes involved in the degradation of alkanes depending on the plant species of the host, the compartment within plants, as well as at various stages of their growth. The highest level of gene expression was observed in the roots and shoots of the Italian ryegrass. All strains demonstrated the expression of the *CYP153* gene in all plant compartments, which indicates their active role in the degradation of diesel oil in the plant. The highest rate of diesel oil degradation (up to 68%) was displayed by two strains: *E. ludwigii* ISI10-3 and *E. ludwigii* BRI10-9 introduced into the Italian ryegrass or alfalfa.

Genetic information encoding the synthesis of enzymes involved in the degradation of organic compounds is most often located in mobile genetic elements such as plasmids or transposons which enable HTG between bacteria [21]. HTG is the main mechanism by means of which microorganisms acquire new metabolic pathways, thanks to which they adapt quickly to the changes which occur in the environment [74]. It allows for increasing the population and the activity of indigenous endophytic bacteria capable of decomposing organic compounds [1]. Moreover, HTG enables the acquisition of endophytic bacteria with new catabolic pathways and heterologous gene expression, especially when the donor and recipient belong to related species [18, 65]. *Burkholderia cepacia* FX2 is a bacterium showing the capability of toluene degradation, hosting a plasmid carrying a gene encoding catechol 2,3-dioxygenase (*C23O*), a key enzyme in the pathway of monocyclic aromatic hydrocarbon degradation. Introduction of
the FX2 strain into maize (Zea mays) and durum wheat (Triticum durum) positively influenced the growth of these plants and also led to the reduction of evapotranspiration of toluene to the atmosphere. Horizontal transfer of the plasmid with the c23o gene among endophytic bacteria naturally occurring in these plants allowed for increasing the population of bacteria capable of pollutant degradation and promotion of plant growth [74]. In turn, Burkholderia phytofirmans PSIN is an endophyte strain which successfully colonises many plants, stimulating their growth and vitality. The genome of this bacterium consists of two chromosomes and one plasmid equipped, among others, with genes encoding decomposition pathways of many complex organic compounds. This bacterium carries genes encoding enzymes involved in the degradation of aliphatic compounds, such as alkane monooxygenase (alkB) and cytochrome P450 hydroxylase. Its genome also contains at least 15 genes encoding dioxygenases, enzymes involved in aromatic-ring fission, such as catechol 1,2-dioxygenase, catechol 2,3-dioxygenase, 2-nitropropane dioxygenase, and protocatechuate 3,4-dioxygenase. Moreover, an impressive number of GST genes (24 copies), which are components of the operons responsible for the degradation of aromatic compounds, have been found in the genome of this strain [41]. Examples of endophytic bacteria, in whose genomes the presence of genes involved in the degradation of organic pollutants has been found, are shown in Table I.

6. Mechanisms enhancing microbe-assisted phytodegradation

Due to the presence of organic pollutants, the availability of minerals, including nitrogen, phosphorus and iron in the soil may be limited, which leads to limitation of plant growth [27]. The bioavailability of minerals in contaminated soils is one of the main factors limiting the effectiveness of phytoremediation [1]. Among bacterial endophytes promoting the growth of plants, it is possible to find such that have the ability to fix free atmospheric nitrogen (diazotrophic bacteria). Recent studies have shown that some bacterial endophytes capable of degrading organic compounds also demonstrate the capability of fixing free nitrogen [27]. Dashti et al. [9] report that bacteria capable of degrading organic pollutants and fixing free nitrogen increase the degradation of hydrocarbons in soils contaminated with petroleum and low in nitrogen. Bacterial endophytes are also able to synthesize specific organic compounds, siderophores, which enable the reduction of Fe$^{3+}$ to Fe$^{2+}$ ions, more easily available to plants [27]. Another macro-component, important for the growth and development of organisms is phosphorus [30]. Bacteria associated with plants may increase the solubility of inorganic phosphates through the synthesis of organic acids and phosphatases [27]. In addition to the minerals discussed above, endophytic bacteria supply essential vitamins [60] to plants, including vitamin B1 [39] and B12 [67].

<table>
<thead>
<tr>
<th>Bacterial endophyte</th>
<th>Host plant(s)</th>
<th>Gene</th>
<th>Coded enzyme</th>
<th>References</th>
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<tbody>
<tr>
<td>Pseudomonas sp. ITR53</td>
<td>Lolium multiflorum L., Lotus corniculatus</td>
<td>alkB</td>
<td>alkane monooxygenase</td>
<td>[4] [51]</td>
</tr>
<tr>
<td>Pseudomonas mandelli 6FXS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhodococcus fascians L11</td>
<td>Lolium multiflorum L., Oenothera biennis</td>
<td>alkH</td>
<td>alkane hydroxylase</td>
<td>[29] [51]</td>
</tr>
<tr>
<td>Rhodococcus sp. 4WK</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterobacter ludwigii IS10-3</td>
<td>Lolium multiflorum var. Taurus, Lotus corniculatus var. Leo, Medicago sativa var. Harpe, Oenothera biennis</td>
<td>CYP153</td>
<td>cytochrome P450 hydroxylase</td>
<td>[82] [51]</td>
</tr>
<tr>
<td>E. ludwigii BR110-9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stenotrophomonas maltophilia 5XS</td>
<td></td>
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<td></td>
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<td>Pseudomonas umsongensis 8.1WK</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Burkholderia cepacia FX2</td>
<td>Zea mays, Triticum durum</td>
<td>c23o</td>
<td>catechol 2,3-dioxygenase</td>
<td>[74]</td>
</tr>
<tr>
<td>Pseudomonas putida VM1441 (pNAH7)</td>
<td>Pisum sativum</td>
<td>ndoB</td>
<td>naphthalene dioxygenase</td>
<td>[15]</td>
</tr>
<tr>
<td>Pseudomonas fluorescens ATCC 17397</td>
<td>Elymus angustus Trin.</td>
<td>nldA</td>
<td>naphthalene dioxygenase</td>
<td>[54]</td>
</tr>
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<td>Pseudomonas syringae Lz4W</td>
<td>Agropyron elongatum</td>
<td>phnAc</td>
<td>phenanthrene dioxygenase</td>
<td>[54]</td>
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<tr>
<td>Pseudomonas poae DSM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhodococcus fascians L11</td>
<td>Lolium multiflorum L.</td>
<td>pah</td>
<td>alpha subunit of the PAH-ring hydroxylating dioxygenases</td>
<td>[29]</td>
</tr>
<tr>
<td>Pseudomonas sp. BF1-3</td>
<td>Platycodon grandiflorus</td>
<td>ophB</td>
<td>organophosphorus hydrolase</td>
<td>[44]</td>
</tr>
<tr>
<td>Burkholderia cepacia VM1468</td>
<td>Populus deltoides x(trichocarpa x deltoides)</td>
<td>tomA</td>
<td>toluene monooxygenase</td>
<td>[70]</td>
</tr>
<tr>
<td>Burkholderia phytofirmans PSIN</td>
<td>Zea mays</td>
<td>GST</td>
<td>glutathione-S-transferase</td>
<td>[41]</td>
</tr>
</tbody>
</table>

Table I

Examples of endophytic bacteria, in whose genomes the presence of genes involved in the degradation of organic pollutants has been found.
One of the most important phytohormones produced by bacterial endophytes is indole-3-acetic acid (IAA), belonging to the group of auxins. This phytohormone stimulates root growth, activates cell division, and induces formation of lateral roots [56]. In addition, IAA can serve as a signal molecule in the communication between bacterial cells [18]. Bacterial IAA also indirectly impacts the regulation of ethylene level in the plant. It may increase the transcription and activity of the plant enzyme of ACC synthase, catalysing the formation of 1-aminocyclopropane-1-carboxylic acid (ACC), a direct precursor of ethylene [17]. Research by Sheng et al. [65] conducted on Enterobacter sp. 12J1 isolated from garlic (Allium macrostemon), growing in areas contaminated with hydrocarbons, showed that this strain is not only capable of degrading pyrene, but also of producing IAA. In addition, it possesses the ability to produce siderophores and solubilise inorganic phosphorus. After inoculation of wheat and maize with the Enterobacter sp. 12J1 strain, it was found that it effectively colonises internal tissues of roots and shoots and the rhizosphere of the studied plants. Plants inoculated with this strain were characterised by increased biomass production.

Ethylene (C$_2$H$_4$) is an important phytohormone, which modulates the growth and metabolism of plant cells. However, its surplus, produced by plants in response to environmental stress, may inhibit root growth, lateral root formation and root hairs [30]. In the ethylene biosynthesis pathway, S-adenosylmethionine (SAM) is converted by ACC synthase to 1-aminocyclopropane-1-carboxylic acid (ACC), which is then converted to ethylene by ACC oxidase. Bacteria exhibit the capability of synthesising ACC deaminase, an enzyme which catalyses the decomposition of ACC into ammonia and α-ketobutyric acid, thus lowering the level of ethylene in the plant [17]. Recent studies have shown that bacterial endophytes, which both produce ACC deaminase and exhibit the capability of hydrocarbon degradation, significantly contribute to the production of plant biomass, as opposed to bacteria that have only one of these features [27]. For example, Yousaf et al. [82] demonstrated a positive correlation between the activity of ACC deaminase present in *E. ludwigii* IS10-3 and *BRI10-9* endophytic strains and the improvement in the efficiency of plant root growth inoculated with these isolates. The examined strains successfully colonised the inside of the roots and the rhizosphere of the plants. In turn, studies by Afzal et al. [2] showed that *Burkholderia phytofirmans* PsJN isolated from surface sterilised onion roots (*Allium cepa*), after introduction into acacia (*Acacia amplusce)* showed a high activity of ACC deaminase, which stimulated plant growth and biomass production in soil contaminated with textile sewage.

Bacterial endophytes may also indirectly stimulate plant growth in soils contaminated with organic compounds by inhibiting the growth and the activity of phytopathogens, induction of the plant resistance mechanisms, synthesis of hydrolytic enzymes, and the distribution of organic pollutants [27].

Many bacterial endophytes exhibit antagonistic effect against numerous fungi and bacteria [56]. They can inhibit the activity of enzymes or degrade toxins produced by pathogens [75]. Some of them are capable of producing lytic enzymes degrading fungal cell walls [39], others produce hydrogen cyanide [7, 29, 50] or many secondary metabolites, including antibiotics [56]. Research by Bisht et al. [7] demonstrated that the endophytic strain of *Bacillus* sp. SBER3 isolated from the poplar (*Populus deltoides*) is able to synthesise IAA, ACC deaminases and siderophores. Moreover, it inhibits under *in vitro* conditions, the growth of phytopathogenic fungi: *Rhizoctonia solani*, *Macrophomina phaseolina*, *Fusarium oxyspotum* and *Fusarium solani* with 60%, 61.5%, 64.3% and 12% respectively. In addition to promoting plant growth, this strain is also capable of degrading polycyclic aromatic hydrocarbons. It was found that after re-inoculation of this strain to the plant, it contributed to the growth of the roots and shoots, the growth of the trunk perimeter, both in the control soil and in the soil contaminated with hydrocarbons [7]. Table II presents examples of endophytic bacteria promoting the growth of plants and exhibiting the ability to degrade organic pollutants.

Some endophytic bacteria useful in phytoremediation are able to produce surface-active agents – biosurfactants [12, 29, 48, 83]. These intracellular, extracellular metabolites or ones associated with the cell wall of microorganisms can be divided into two groups. The first group includes compounds of relatively low molecular weight (glycolipids, phospholipids, lipopeptides), the second group is formed by high molecular weight substances, such as emulsan, liposan or polysaccharide-protein complexes. Low molecular weight biosurfactants generally strongly reduce interphase and surface tension. On the other hand, high molecular weight biosurfactants are mostly effective emulsifiers [47, 48]. The biosurfactants, as well as microorganisms that produce them, play a special role in the bioremediation of environments contaminated with petroleum hydrocarbons [32]. The remediation of such environments is limited due to the high hydrophobicity of this type of contaminants and their low solubility in water. These compounds are adsorbed on soil particles or trapped in soil pores and thus become inaccessible to microorganisms and plants [12, 83]. The presence of biosurfactants produced by microorganisms contributes to the increased bioavailability of organic pollutants and facilitates their uptake by plants [12]. By
interacting directly with hydrocarbons, biosurfactants increase their solubility in water, and by interacting with the surface of bacterial cells degrading these substances, they contribute to their hydrophobicity [5, 12]. Moreover, the presence of biosurfactants in the environment may impact both individual cells and entire microbial populations. For example, some cyclic lipopeptides produced by the bacteria of the genus Bacillus and Pseudomonas, apart from very high surface activity, are characterised by biological activity – inhibiting the growth of phytopathogens, they contribute to activating induced systemic resistance (ISR), participate in the formation of biofilms or in swarming motility [46, 57]. The ability to produce biosurfactants has been demonstrated in such endophytic strains as Pseudomonas sp., Microbacterium sp. and Kocuria sp. isolated from Lolium perenne [29], Bacillus subtilis K1 strain isolated from the roots of banyan fig (Ficus benghalensis L.) [48] and Microbacterium sp. M87 derived from the water hyacinth tissues (Eichhornia crassipes) [32]. Research by Wu et al. [79] proved that Pseudomonas aeruginosa L10 strain, isolated from the roots of the reed (Phragmites australis), producing a biosurfactant which effectively reduces surface tension, shows high degradation activity towards various hydrocarbons. Sequencing the genome of this strain showed the presence of rhlABRI genes encoding enzymes involved in the biosynthesis of rhamnolipid, one of the most commonly studied surface-active agents. In turn, in 2014, Zhang et al. [83] demonstrated that inoculation of plants with a bacterial consortium containing an endophytic B. subtilis J4A strain capable of diesel oil degradation and the endophytic Pseudomonas sp. U-3 strain producing a biosurfactant which effectively reduces surface tension, promoted the removal of diesel oil from the environment. Biosurfactants produced by endophytes can be a good alternative to the increasingly controversial synthetic surface-active agents used to support bioremediation processes [31]. The use of synthetic surfactants raises concerns, especially due to their toxicity and resistance to biodegradation, which may lead to an increase in environmental pollution with this type of compounds [8, 23, 42]. The ability to degrade anionic surfactants has been demonstrated in soil bacteria B. subtilis and Bacillus cereus [49] and in bacteria of the genus Pseudomonas isolated from soil and water [58]. Synthetic surfactants may also adversely impact the chemical and physical structure of soil and accumulate in groundwater [61].

<table>
<thead>
<tr>
<th>Bacterial endophyte</th>
<th>Host plant(s)</th>
<th>Plant growth-promoting traits</th>
<th>Degraded organic pollutants</th>
<th>References</th>
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<tbody>
<tr>
<td>Pseudomonas putida L1</td>
<td>Lolium perenne L.</td>
<td>production of IAA, production of siderophores, production of HCN</td>
<td>diesel oil</td>
<td>[29]</td>
</tr>
<tr>
<td>Enterobacter asburiae 4FJK</td>
<td>Hieracium piloselloides</td>
<td>production of ACC deaminase, production of IAA, production of HCN, production of NH3</td>
<td>diesel oil</td>
<td>[50]</td>
</tr>
<tr>
<td>Pseudomonas sp. 1FWK</td>
<td>Oenothera biennis</td>
<td>production of ACC deaminase, production of IAA, production of siderophores</td>
<td>diesel oil</td>
<td>[51]</td>
</tr>
<tr>
<td>Enterobacter ludwigii IS10-3, E. ludwigii IS10-4, E. ludwigii BR110-9</td>
<td>Lolium multiflorum var. Taurus, Lotus corniculatus var. Leo, Medicago sativa var. Harpe</td>
<td>production of ACC deaminase</td>
<td>alkanes</td>
<td>[82]</td>
</tr>
<tr>
<td>Bacillus sp. SRER3</td>
<td>Populus deltoides</td>
<td>production of ACC deaminase, production of IAA, production of siderophores, production of lytic enzymes</td>
<td>anthracene, naphthalene, benzene, toluene, xylene</td>
<td>[7]</td>
</tr>
<tr>
<td>Enterobacter sp. 12J1</td>
<td>Allium macrostemon</td>
<td>production of IAA, production of siderophores, phosphate solubilization</td>
<td>pyrene</td>
<td>[65]</td>
</tr>
<tr>
<td>Achromobacter xylosidans F3B</td>
<td>Chrysopagon zizanioides</td>
<td>production of IAA, nitrogen fixation</td>
<td>toluene</td>
<td>[19]</td>
</tr>
<tr>
<td>Burkholderia cepacia FX2</td>
<td>Zea mays, Tricticum durum</td>
<td>production of siderophores, phosphate solubilization</td>
<td>toluene</td>
<td>[74]</td>
</tr>
<tr>
<td>Burkholderia phytofirmans PSIN</td>
<td>Acacia amplexic</td>
<td>production of ACC deaminase</td>
<td>textile wastewaters</td>
<td>[2]</td>
</tr>
<tr>
<td>Pseudomonas sp. E46</td>
<td>Spirodela polyrhiza</td>
<td>production of IAA</td>
<td>fenpropathrin</td>
<td>[75]</td>
</tr>
</tbody>
</table>

Table II
Examples of endophytic bacteria promoting the growth of plants and exhibiting the ability to degrade organic pollutants
ENDOPHYTIC BACTERIA IN THE PHYTODEGRADATION OF PERSISTENT ORGANIC POLLUTANTS

7. Summary

The use of endophytic bacteria in the remediation of organic pollutants is currently one of the most important research directions in environmental biotechnology. Numerous studies confirm that the relationship between plants and their internal symbiotes clearly contributes to the effective course of bioremediation. Therefore, it is worth exploring these interactions in order to develop methods and strategies to make better use of these interactions. Newly isolated endophytic bacteria may become a starting point for further studies on their ability to degrade organic pollutants or support plant growth. Identification and understanding of the genetic basis of interactions between plants and endophytes, using the latest molecular biology techniques, will allow the full potential of this group of microorganisms to be exploited.

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References


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