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Review

Simply Versatile: The Use of *Peribacillus simplex* in Sustainable Agriculture

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Abstract: *Peribacillus simplex* is a Gram-positive, spore-forming bacterium derived from a vast range of different origins. Notably, it is part of the plant-growth-promoting rhizobacterial community of many crops. Although members of the *Bacillaceae* family have been widely used in agriculture, *P. simplex* has, so far, remained in the shadow of its more famous relatives, e.g., *Bacillus subtilis* or *Bacillus thuringiensis*. Recent studies have, however, started to uncover the bacterium's highly promising and versatile properties, in particular in agricultural and environmental applications. Hence, here, we review the plant-growth-promoting features of *P. simplex*, as well as its biocontrol activity against a variety of detrimental plant pests in different crops. We further highlight the bacterium's potential as a bioremediation agent for environmental contaminants, such as metals, pesticide residues, or (crude) oil. Finally, we examine the recent developments in the European regulatory landscape to facilitate the use of microorganisms in plant protection products. Undoubtedly, further studies on *P. simplex* will reveal additional benefits for agricultural and environmentally friendly applications.

Keywords: *Peribacillus simplex*; antimicrobial activity; sustainable agriculture; bioremediation; European Green Deal

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1. Introduction

Sustainable agriculture is key in ensuring a continuous food supply for the growing world population, while at the same time minimizing negative effects on the environment [1]. This is also reflected in policy developments such as the European Green Deal and its ambitious Farm to Fork strategy, aiming at halving the use and risk of chemical pesticides and increasing organic farming practices [2].

One promising approach to replacing chemical products is the use of soil microbial inoculants, which are predominantly based on plant-growth-promoting (PGP) fungi and bacteria [3,4]. When applied to soil and/or plants, these microorganisms can exert several beneficial effects on their surroundings, such as (i) acting as biopesticides, (ii) enhancing plant growth, or (iii) improving soil conditions (e.g., through bioremediation or bioadsorption) [3]. Thus, bacterial inoculants can increase agronomic efficiency by reducing production costs and environmental pollution, as well as (partially) eliminating the use of chemical fertilizers and pesticides [5].

Plant-growth-promoting bacteria (PGPB) and plant-growth-promoting rhizobacteria (PGPR) are essential parts of the soil microbiome, sustaining plant health and growth. These microorganisms colonize the soil, plant rhizosphere, and root surface or interior and fulfil a variety of useful functions, such as increasing nutrient availability, counteracting abiotic stress, or improving the stress tolerance of the plant [5–8]. Here, members of the genus *Bacillus*—and recently reclassified closely related genera [9]—are one of the predominant microbial communities and play an important role in maintaining healthy soils conducive for plant growth and nutrition [8,10]. These Gram-positive bacteria are characterized by their ability to form dormant endospores, enabling them to withstand harsh conditions otherwise fatal to vegetative cells [11–13]. In addition, their ability to produce a wide arsenal of biologically active compounds with inhibitory and/or plant-growth-promoting effects has been well documented [14–16].

The biocontrol activity of a microorganism can generally be classified into two mechanisms. Direct antimicrobial activity includes the synthesis of phytohormones, as well as the production of antibiotics, hydrolytic enzymes, or lipopeptides [17]. In this regard, *Bacillus* spp. have been recognized as promising sustainable plant protection agents presenting a viable alternative to chemical pesticides [18], with, e.g., *B. thuringiensis*-, *B. subtilis*-, and *B. amyloliquefaciens*-containing formulations already commercially available [17,19,20].

Indirect mechanisms of biocontrol activity include (amongst others) inducing systemic resistance (ISR) in plants [17], which activates/increases plants' resistance towards phytopathogenic infections and indirectly stimulates plant growth [21,22]. Here, *Bacillus* spp. can induce systemic resistance through different mechanisms, such as the secretion of enzymes, cyclic lipopeptides, or volatile organic compounds (VOC) [23]. That said, it is important to note that there is no clear separation of ISR and antimicrobial activity, as several antimicrobial lipopeptides, e.g., fengycin and surfactin, or VOCs can simultaneously induce systemic resistance [24].

Although positive environmental impacts of members of *Bacillus* spp. and related genera have been widely demonstrated, studies on *Peribacillus simplex* have only recently started to uncover the bacterium's wide range of highly promising PGP features, including the ability to promote plant growth through nutrient fixation, the production of antimicrobial compounds, or acting as biosorbent for environmental contaminants. Hence, here, we provide a comprehensive overview of these findings and highlight *P. simplex*'s potential for its use in sustainable agricultural. Finally, with a view towards the future applications of *P. simplex* as a biocontrol agent, we will briefly summarize the requirements and changes in the European Regulation in the light of the European Green Deal and Farm to Fork strategy, which aim to facilitate the use of microorganisms in plant protection products.

2. Genus Peribacillus

Members of the genus *Peribacillus* belong to the family of *Bacillaceae* and are rod-shaped, Gram-positive, endospore-forming bacteria. Aerobic or facultative anaerobic bacteria were previous members of the genus *Bacillus*, however, after an extensive taxonomic reclassification in 2020 using phylogenomics and comparative genomic analyses, the species have been rearranged based on molecular markers to form a separate monophylogenetic group of the genus *Peribacillus* [9,25]. Today, the genus includes 21 species, with *Peribacillus simplex* as the type strain (Table 1) [26].

Many of the species have been originally isolated from soil and plant samples, although they can be derived from a wide variety of origins, such as near the Viking spacecraft at Kennedy Space Center [27] or stratospheric air samples at a 41 km altitude [28].

Table 1. Members of the *Peribacillus* genus. Original sources of isolation are indicated.

***Peribacillus* Species [25,26]**	**Original Isolation Source**	**Ref.**
Peribacillus acanthi	Rhizosphere soil of a mangrove plant *Acanthus ilicifolius*	[29]
Peribacillus alkalitolerans	Marine sediment near a hydrothermal vent	[30]
Peribacillus asahii	Soil	[31]
Peribacillus butanolivorans	Soil	[32]
Peribacillus castrilensis	River otter	[33]
Peribacillus cavernae	Cave soil	[34]
Peribacillus deserti	Desert soil	[35]
Peribacillus endoradicis	Soybean root	[36]
Peribacillus faecalis	Cow feces	[37]
Peribacillus frigoritolerans	Arid soil	[38,39]

Table 1. *Cont.*

Peribacillus **Species [25,26]**	**Original Isolation Source**	**Ref.**
Peribacillus glennii	Vehicle assembly building at Kennedy Space Center	[27]
Peribacillus gossypii	Stem of *Gossypium hirsutum*	[40]
Peribacillus huizhouensis	Paddy field soil	[41]
Peribacillus kribbensis	Soil	[42]
Peribacillus loiseleuriae	Soil from a loiseleuria plant	[43]
"*Peribacillus massiliglaciei*" [1]	Siberian permafrost	[44]
Peribacillus muralis	Deteriorated mural paintings	[45]
Peribacillus psychrosaccharolyticus	Soil or lowland marsh.	[46]
Peribacillus saganii	Vehicle assembly building at Kennedy Space Center	[27]
Peribacillus simplex	Soil	[46]
Peribacillus tepidiphilus	Tepid spring	[47]

[1] Nomenclature status not validly published.

3. Plant-Growth-Promoting Properties

Members of the *Bacillus* genus (as traditionally defined) are among the most widespread Gram-positive soil microorganisms and are predominant in the plant-growth-promoting bacteria (PGPB) community [10]. The beneficial effects of the family members have been well documented [8,10,18].

In this regard, a number of studies have highlighted *P. simplex*'s potential to act as a plant-growth-promoting microorganism (Table 2).

Table 2. Examples of uses of *Peribacillus simplex* as plant-growth-promoting bacteria.

P. simplex **Isolate**	**Effect**	**Tested Plant**	**Ref.**
MRBN26	Increased shoot and root weight	Canola plant	[48]
KY604953	Enhanced germination, root growth, and nutrient uptake	Wheat	[49]
K10	Improved plant height, tuber weight, photosynthesis yield, transpiration rate, water use efficiency, and overall yield	Potato	[4]
MH671854.1, MH671861.1	Increased shoot and root weight, IAA production, and high phosphate solubilization	Tomato	[50]
KBS1F-3	Increased shoot and root weight, IAA production, and high phosphate solubilization	Tomato and wheat	[51]
KY515398	Stimulation of root and shoot growth	Corn, wheat, and soybean	[22]
L266	Stimulation of primary root growth and lateral root development	*Arabidopsis thaliana*	[52]
30N-5	Increases number of lateral roots	Pea legume	[53]
PHYB1; PHYB9	Increased root and foliar dry weight	Black cumin	[54]
313, 371	Increased phosphate uptake and increased soil nutrient concentrations (co-cultured with *P. biliaiae*)	Winter wheat	[55]
RC19	Root induction	Kiwi	[56]
SYM00260	Increased yield and root and shoot dry weight	Corn and soybean	[57]
UT1	Improved phosphate, potassium, and silica uptake, and increased root and shoot biomass	Wheat	[58]
EGE-B-1.2.k	High phosphate solubilization	Tomato, pepper, and eggplant	[59]
499G2	Increased nitrogen, phosphorus, And IAA in plant leaves	Wild rice	[60]

3.1. Plant Growth Promotion through Compound Secretion

With the aim of searching for sustainable plant supplements or alternatives to chemical fertilizers, the use of PGPB has shown great potential, minimizing environmental impacts [51]. *P. simplex* demonstrates a broad range of activity, stimulating growth in a large variety of commercially relevant crops, such as tomato, wheat, soybean, or corn (Table 2), and has sometimes achieved over a quarter of crop yield increase [57]. In some cases, growth stimulation can notably reach levels similar to chemical fertilizers, making the bacterium a sustainable alternative to potentially harmful chemicals in food production [50]. Growth stimulation has most commonly been attributed to direct growth promotion via auxin production (indole-3- acetic acid, IAA) or siderophore secretion [22,50,56,58].

Another way of stimulating plant growth is the emission of volatile organic compounds (VOC), e.g., acetoin and 2,3-butanediol. When emitted by PGPB bacteria, these compounds can act as plant growth promotion triggers [52]. Gutiérrez-Luna et al. suggested that the VOCs secreted by *P. simplex* isolated from lemon plants improved the root growth and development in *Arabidopsis thaliana* under greenhouse conditions [52]. These compounds, mostly ketones and aldehydes also with antimicrobial attributes, included 2-nonenal, benzaldehyde, acetophenone, 6,10,14-trimethyl-2-pentadecanone, and 1-butanol, amongst others. However, there was no direct, experimental support for the effect of specific VOCs on plant growth promotion [52].

Finally, recent studies have shown that these growth promotion effects can be maximized when using combined inoculations with other PGPBs [51] or inorganic material [58]. This effect was particularly visible when combining PGP bacteria (*P. simplex*) and nitrogen (N)-fixating rhizobacteria (*B. subtilis*, *Rhizobium leguminosarum* bv. *Viciae*) in peas [53], while *P. simplex*-based bioformulations showed hydrogen cyanide (HCN), siderophore, and ammonia production in wheat [49]

In contrast, studies investigating the addition of inorganic acids such as salicylic acid together with *P. simplex* did not show any effect on plant growth [61].

3.2. Improved Nutrient Availability

Recent research attempts have aimed at increasing the concentrations of specific nutrients or micronutrients, thus improving plant health and nutritional value [4]. Although many techniques are based on plant-breeding techniques or transgenics, the use of PGP bacteria could also boost the uptake of specific nutrients in crops.

Studies have shown that siderophore-producing *P. simplex* can increase the uptake of iron in potatoes, while at the same time improving overall plant growth and yield [4].

P. simplex isolates have also demonstrated a high phosphate and zinc solubilization index in wheat [49], whereas high phosphate solubilization was detected in experiments with tomato plants. The latter, however, was distinctly strain-dependent [50].

Given that, in the soil, microorganisms occur in communities presumably acting synergistically, the combination of several PGPBs has shown better plant growth promotion effects than when used in isolation [3]. For example, co-culturing canola plants with *P. simplex* improved the shoot and root weight, in addition to enhancing the molybdenum micronutrient uptake [48]. Higher soluble nutrient concentrations (phosphate, magnesium, manganese, and sulfur), as well as increased phosphate uptake, could be obtained in winter wheat upon co-inoculation of the soil fungus *Penicillium bilaiae* with *P. simplex* (isolated from *P. biliaiae*) [55]. Equally, co-culturing *P. simplex* with inorganic silicon (Si) could improve the phosphate (P) uptake from P-rich and P-deficient soils. This was attributed to reduced oxidative stress as a result of increased antioxidant enzyme production, ultimately lowering the environmental stress for the plant and preventing root deterioration.

3.3. Root Colonization

PGPRs colonize the soil closely surrounding plant roots (rhizosphere), where they exert beneficial effects on plants. Hence, the success of microorganisms used as inoculants in agricultural crops greatly depends on the ability to colonize the host plant roots and body and prevail against other competing microorganisms [5,62]. The successful association of the bacteria with the plant roots is achieved by chemotaxis, attachment, and distribution along the roots. Once established, the bacterial colony size will determine and improve the root coverage and antagonism [62].

P. simplex has demonstrated a good root colonization potential and persistence in several commercial plants, such as wheat, tomato, and pine tree roots [51,55,62]. In some cases, *P. simplex* showed a higher rate of colonization than other *Bacillus* species (e.g., *B. subtilis*) [49]. Fluorescent localization studies with the transgenic *P. simplex* strain S11R41 isolated from pine tree rhizosphere have, in particular, confirmed that the bacterium is able to rapidly associate with tree roots, forming clusters at emerging lateral roots and elongation zones [62].

Regarding biofilm formation, GFP-report localization studies have not evidenced any biofilm formation of *P. simplex* associated with tree roots [62].

4. Biocontrol Activity

P. simplex strains isolated from different environments showed biocontrol activity against a large range of phytopathogens, mostly fungi, but also nematodes and bacteria, which was detected in several commercially highly relevant plants, such as potato, wheat, or tobacco (Table 3).

Table 3. Applications of *Peribacillus simplex* as biocontrol agent in selected crops/diseases and associated phytopathogens. Studies on the species' antimicrobial activity, as well as the induction of the plant systemic response, are considered.

P. simplex Isolate	Effect	Target	Class	Test Conditions (Plant) *	Ref.
		Antimicrobial activity			
30N-5; 11; 237	Presence of biocontrol genes/cellulase, xylanase, pectinase, and chitinase production	*Fusarium* spp.	Fungus	In vitro/In silico	[17]
30N-5	Pathogenetic growth inhibition			In vitro	[53]
R180	Pathogenetic growth inhibition and reduction in disease severity			In vitro and in planta (corn, wheat, and soybean)	[63]
PHYB1 and PHYB9	Reduction in disease severity, and hyphal tissue maceration			In vitro and in planta (black cumin)	[54]
Isolate 1–6	VOC production	*Panagrellus redivivus* and *Bursaphelenchus xylophilus*	Nematode	In vitro	[64]
Alg.24B2	Production of lytic enzymes and lipopetides	*Zymoseptoria tritici*	Fungus	In vitro	[65]
03WN13; 03WN23;03WN25	Reduced lesion size and disease (pink rot)	*Phytophthora erythroseptica*	Fungus	In planta (potato)	[66]
BA2H3	Pathogenetic growth inhibition and reduction in soft rot symptoms	*Pectobacterium* sp.	Bacterium	In vitro and in planta (potato)	[67]
UJA_MA_369	Pathogenetic growth inhibition	*Xylella fastidiosa*	Bacterium	In vitro	[68]
		Induced Systemic Resistance			
HS-2	Antifungal/ increased ROS and callose production	*Pythium aphanidermatum*	Phytium	In vitro and in planta (tobacco)	[23]
499G2	Increased antioxidant enzyme production	*Magnaporthe grisea*	Fungus	In vitro and in planta (wild rice)	[60]
S11R41	Reduced lesions and plant mortality	*Heterobasidion annosum* and *Armillaria mellea*	Fungus	In vitro and in planta (*Pinus radiata*)	[69]
	Reduced fungus growth and density and reduced lesion length	*Fusarium circinatum*			[70]
Sneb545	Increased plant resistance, reduced infection/nematode penetration, and reduced nematode growth	*Heterodera glycines*	*Nematode*	In vitro and in planta (soybean seeds)	[71–73]

* Test conditions indicate if studies were performed in vitro, in silico, or the tested plant in case of in planta tests.

4.1. Antimicrobial Activity

The antifungal activity of *P. simplex* has been demonstrated in a number of studies, most of them conducted on the phytopathogenic fungus *Fusarium* spp. In vitro assays showed up to a 70% growth inhibition of the plant pest and fungal hyphal thinning [17,53,63], however, compared to *B. subtilis*, the effects were slightly lower [17]. In planta experiments further confirmed these antifungal properties, greatly reducing disease severity after *P. simplex* application to the root seedlings of row crops or in black cumin [54,63]. The authors cautioned, however, that the results obtained from in vivo and in vitro antagonistic assays were not always aligned [63], and thus appropriate care should be taken for the screening of biocontrol agents under field conditions. Schwartz et al. [53] also confirmed *P. simplex*'s antagonistic activity against *Fusarium* spp., which was, however, dependent on growth conditions. This study was of particular interest, as it demonstrated the combined antimicrobial and plant-growth-promoting effects of *P. simplex* isolate 30N-5 in pea (Tables 2 and 3), suggesting that such a combined activity could be more effective under field conditions [53]. Similar results were observed for isolates PHYB1 and PHYB9 in black cumin treatment [54]. Regarding the mode of action, in silico genomic studies indicated the presence of genes involved in the chitin degradation pathway and hydrolytic enzyme production, as well as cell-wall-degrading enzymes such as cellulase, pectinase, and xylanase, all of which are indicators for *P. simplex*'s antimicrobial activity [17]. Scanning electron microscopy studying the interaction between *P. simplex* and *F. camptoceras* demonstrated the bacterial adhesion to the fungus and the colonization of hyphae, causing tissue maceration [54].

P. simplex also reduced fungi-associated diseases in potato (pink rot) and wheat (Septoria Tritici Blotch) [65,66], while other studies demonstrated its antagonistic activity against the phytopathogens *Pectobacterium* sp. and *Xylella fastidiosa* [67,68]. Finally, in silico studies of the strain BA2H3 suggested the production of the antimicrobial compounds bacitracin and anthrachelin [67,74].

Regarding VOCs, several studies have highlighted *P. simplex*'s ability to produce a variety of microbial volatile organic compounds, including 2-ethyl-3,5-dimethylpyrazine, phenol, 1-decanol, 2-propanone, and benzaldehyde [17,64]. In this regard, Gu et al. [64] showed that soil-derived *P. simplex* strains secreted a mix of volatile organic compounds from the phenol, alcohol, aldehyde ketone alkyl, alkene, acid, ether, or heterocyclic groups, with strong antagonistic activity against the parasitic nematodes *Panagrellus redivivus* and *Bursaphelenchus xylophilus*. One important consideration with regard to the use of bacterial VOCs is that this mix is potentially less likely to select for resistance upon fumigation treatment.

4.2. Systemic Resistance

Recent studies have indicated that, besides antifungal activity in tobacco plants, pretreatment with the *P. simplex* strain HS-2 increased reactive oxygen species (ROS) production and lowered plant cell wall permeability through increased callose production in response to a pathogen challenge [23]. Both reactions are indicators of the plant immune response. In addition, priming with this strain enhanced the expression of plant-related defense genes (e.g., lipoxygenase), as well as MAPK (mitogen-activated protein kinases) signals [23].

Fungal antagonism was also demonstrated in vitro and in planta against the forest fungal pathogens *Heterobasidion annosum* s.s., *Armillaria mellea*, and *Fusarium circinatum*. Notably, the treatment of pine seedlings with *P. simplex* considerably reduced lesions and plant mortality after pathogen exposure, which was tentatively attributed to antibiosis/systemic response [69,70]. Here, a dual application of the bacterium together with essential oils able to reduce seedling lesions was suggested as a plant prophylactic treatment [70].

Several studies by Yu-xi Duan and colleagues furthermore demonstrated the antagonistic effects of *P. simplex* Sne545 against nematodes through the activation of induced systemic resistance in soybean using a wide range of different analytical approaches [71–73]. First, metabolomic and transcriptomic analyses showed that the bacterium induces ISR by modulating the accumulation of nematocidal compounds (4-vinylphenol, L-methionine, piperine, and palmitic acid) after root infection, hence improving soybean resistance against pathogenic attacks [72]. Then, additional ISR-active compounds were determined using ^{1}H-NMR and ^{13}C-NMR as cyclic (Pro-Tyr), phenylalanine, cyclic (Leu-Pro), uracil, cyclic (Val-Pro), and tryptophan. The latter three notably activated the root resistance pathways (SA and JA pathways) in the plant [71]. Finally, metabolomics studies identified 15 metabolites involved in nematode resistance as a result of *P. simplex* Sne545 priming.

These metabolites were involved in the provision of nematode nutrient sources (glucose, fructose, sucrose, and trehalose), the production of nematocidal compounds (melibiose and gluconic acid, lactic acid, phytosphingosine, and noradrenaline), and improved disease resistance (oxoproline, maltose, and galactose) [73].

Studies on wild rice have furthermore highlighted that pretreatment with strain 499G2 can promote plant growth (mostly through IAA production), while at the same time inducing plant resistance [60]. Overall, this is a good illustration that systemic resistance in plants (and bacterial antagonistic activity) mostly consists of an elaborate interplay of different pathways and compounds warding off the phytopathogen and often simultaneously improving plant resistance, survival, and health [60,71–73].

5. Biosorption and Bioremediation

The use of microorganisms as a remedy for contaminated zones is widely accepted. Several microorganisms have shown good potential as biosorbents for binding metals, environmental contaminants, or even mineral oil, immobilizing the contaminating substances and hindering their entry into the plant, food chain, or ground water [75–77]. In addition, bioremediation by microorganisms can indirectly promote plant growth by reducing stress conditions. In this regard, studies throughout the years have shown the bioremediation activity of *P. simplex* (Figure 1).

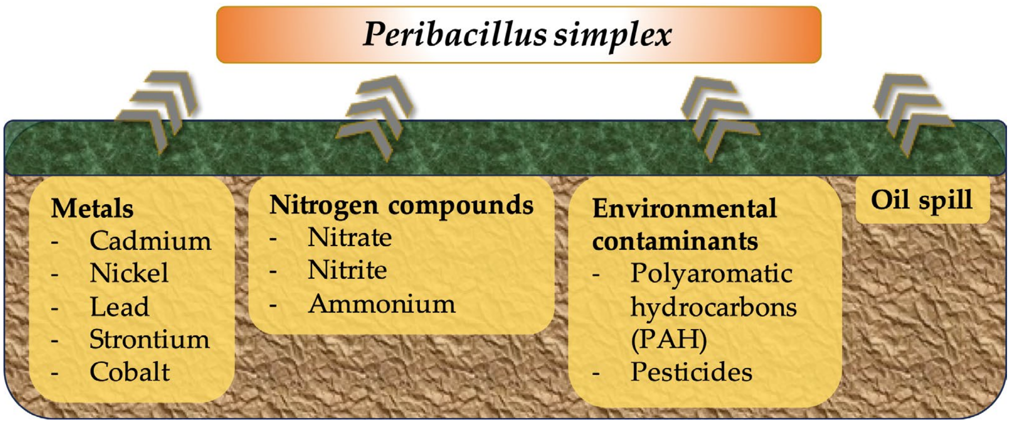

Figure 1. Schematic representation of bioadsorption and bioremediation activity of *P. simplex*.

Early studies performed in the 1990s revealed that *P. simplex* could remove metals from contaminated soils and thus act as an environmental decontamination agent [75]. The bacterial uptake of cationic metal is usually attributed to interactions with the negatively charged cell wall. In particular, *P. simplex*'s ability to adsorb heavy metals showed a pH dependency with an optimum performance close to a neutral to alkaline pH. Researchers thus concluded that metal uptake was dependent on variably charged protonation sites (e.g., amino groups, phosphate, or carboxylate) [75]. As an example, Valentine and colleagues showed how the *P. simplex* strain ZAN-44 can adsorb divalent cadmium, nickel, cobalt, and strontium ions with a higher efficiency than *B. subtilis* 168 or *Escherichia coli* K-12. Notably, the latter two of the tested ions (^{60}Co and ^{90}Sr) were radionuclides, making *P. simplex* an interesting biosorbent for the cleaning of radioactively contaminated sites [75]. The ability of *P. simplex* to adsorb lead has been demonstrated in the literature, while authors have suggested that the bacterium could be exploited for bioremediation purposes [76]. Elevated levels of cadmium have been a major concern also in cocoa plants, with many initiatives aiming at reducing cadmium levels. Here, *P. simplex* has been proven as a highly promising sustainable biosorbent material for removing cadmium from contaminated soils and preventing its entry into plants and food chains [78].

Bioremediation activity has also been shown for other environmental contaminants such as low-molecular-weight polyaromatic hydrocarbon fluorene and phenanthrene, as well as nitrate, nitrite, and ammonium [79–81]. In particular, nitrogen removal capacity was favored by the strains' (*P. simplex* H-b) tolerance of low temperatures [81].

The pesticidal burden of soils has become an increasing concern in agriculture and the food industry, given the long-time stability and non-specific toxicity of many active substances [82]. In this regard, several studies have demonstrated *P. simplex*'s ability to remove chemical pesticides from contaminated soils, as shown with the example of chlorsulfuron [83].

Finally, *P. simplex* isolates derived from bioaugmented oil contaminated soil have been classified as hydrocarbonoclastic bacteria, i.e., able to live on hydrocarbons as an energy source [84]. In addition to biodegradation, a *P. simplex* strain isolated from oil-contaminated sea sediment showed a high oil recovery efficiency through the production of a lipopeptide surfactant, including at a high salinity [85]. These features make *P. simplex* a particularly interesting candidate for the bioremediation of (crude) oil-contaminated sites via oil degradation and recovery.

6. EU Regulatory Aspects on the Use of Microorganisms in Sustainable Agriculture

Plant pathogens present a serious threat to agricultural productivity and can cause severe crop loss. For decades, chemical pesticides have been used to fight phytopathogens, including bacteria, fungi, or insects. However, with regulatory and food safety requirements becoming much stricter, a switch towards sustainable agriculture using biological alternatives to hazardous chemicals is gaining importance. In this regard, *P. simplex* and other members of the *Bacillaceae* family have shown promising traits that could be exploited in commercial agriculture, thus providing solutions to recent policy requests. Here, with the aim of contributing towards the objectives set under the Farm to Fork Strategy to reduce the overall use and risk of chemical pesticides by 50% and the use of more hazardous pesticides by 50% by 2030 [2], the European Union (EU) is facilitating the application of microorganisms in plant protection products. More specifically, it has developed four implementing regulations—applicable since 2022—regarding the approval of microorganisms as active substances in plant protection products (PPP). The first modification was Commission Regulation (EU) 2022/1438 amending Annex II of Regulation (EC) No 1107/2009 [86,87]. The latter provides rules for the authorization of PPPs and their placing on the market, while the amendment (amongst others) extends specific criteria related to microorganisms. Some of these main modifications and/or additions specifically refer to the requirement that the microorganism in question needs to be deposited at an internationally recognized culture collection and receive an accession number. It must be identified at minimum at the strain level and information must be provided about whether the biological materials are wild types, mutants, or genetically modified organisms. Regarding the safety aspects of the microorganisms, they must not be pathogenic to humans and must have no known functional and transferable gene coding for resistance to relevant antimicrobial agents. In this regard, the amendment further requires the microorganism to be susceptible to at least two classes of antimicrobial agents for it to be considered a low-risk active substance [87].

Other amendments related to the necessary information to be submitted for active substances and the specific data requirements for microorganisms were Commission Regulation (EU) 2022/1439 amending Regulation (EU) No 283/2013 [88,89]. We particularly highlight a modification referring to antimicrobial resistance (AMR), as well as the presence of antimicrobial resistance genes (ARG) [88]. Here, information is required on whether the bacterium shows any resistance to relevant antimicrobial agents or if ARG are acquired, transferable, and functional. These changes also relate to modifications in the data requirements for plant protection products containing microorganisms, as reflected in Commission Regulation (EU) 2022/1440 amending Part B of the Annex to Regulation (EU) No 284/2013 [90,91]. Thus, both amendments aim to update the data requirements for the latest scientific developments and adapt them to the specific biological properties of microorganisms.

Finally, given the abovementioned updated regulatory documents, Commission Regulation (EU) 2022/1441 amends Regulation (EU) No 546/2011 regarding the uniform principles for the evaluation and authorization of plant protection products containing microorganisms. Hence, data assessments are aligned across Member States, ensuring a high level of protection for human and animal health [92,93].

7. Conclusions

The advantages of *Bacillus* spp. in agriculture have long been recognized. That said, *Peribacillus simplex* has not received as much attention as other strains in this regard. However, recent efforts focusing on this spore former have shown its various beneficial effects for agricultural and environmental applications. These notably include plant-growth-promoting properties and excellent root colonization skills, as well as antimicrobial compound production and the induction of the plant systemic immune response. Regarding environmental functions, studies have begun to reveal highly promising properties of *P. simplex* as a bioremediation agent, for example, of heavy metals, pesticides, or oil removal and recovery. Future work will surely uncover further modes of action for this versatile bacterium.

A revision of the European regulatory landscape highlights changes in the legal frameworks to facilitate the use of microorganisms in sustainable plant protection products, while imposing strict safety rules to protect humans, animals, and the environment.

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