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Exploring 2,4-diacetylphloroglucinol: A multifunctional biocontrol and therapeutic potential

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2,4-Diacetylphloroglucinol (2,4-DAPG) is a polyketide-derived secondary metabolite synthesised primarily by fluorescent *Pseudomonas* spp., notably *P. fluorescens* Pf-5, CHA0, and Q2-87. This review comprehensively explores the biosynthetic, genetic, and regulatory frameworks underlying 2,4-DAPG production, along with its broad-spectrum biological activities that bridge agricultural and biomedical significance. Despite extensive documentation of its biocontrol efficacy, research gaps persist regarding its coordinated regulation by the *phl* gene cluster and global networks such as GacS/GacA and small RNAs (RsmXYZ), which influence metabolite flux under environmental cues. Recent studies highlight its potent antimicrobial, antifungal, antibacterial, nematicidal, antihelminthic, phytotoxic, and plant growth-regulating activities mediated through membrane disruption, inhibition of oxidative phosphorylation, and induction of reactive oxygen species. Its ability to trigger induced systemic resistance (ISR) via jasmonic acid and ethylene pathways enhances plant immunity and crop resilience. Beyond agriculture, 2,4-DAPG exhibits pronounced cytotoxic and anticancer potential, demonstrating selective antiproliferative effects against human lung (A549), breast (MCF-7), and leukaemia (HL-60) cell lines, along with notable anti-inflammatory and antiviral properties. The pro-apoptotic mechanism involves Bax activation and caspase-3 signalling, coupled with inhibition of NF-κB, MAPK1, and WNT1 cascades. To meet the rising demand, future research emphasises metabolic engineering of high-yield *Pseudomonas* strains, multi-omics-guided metabolic pathway optimisation, heterologous gene expression, and bioreactor-based fermentation for scalable production. In conclusion, 2,4-DAPG represents a multifunctional metabolite with immense potential for sustainable agriculture and therapeutic innovation through large-scale, eco-efficient biomanufacturing.

Keywords: 2,4-Diacetylphloroglucinol, Anticancer potential, Antimicrobial activity, Induced systemic resistance, Sustainable agriculture

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Introduction

Fluorescent *Pseudomonas* species are ubiquitous in both temperate and tropical soils and often dominate the rhizosphere of diverse crop plants. These bacteria promote plant growth through a dual mechanism: directly by synthesizing phytohormones that stimulate root and shoot development, and indirectly by secreting a broad range of bioactive secondary metabolites that enhance nutrient availability and suppress phytopathogens. In addition to nutrient solubilization, they produce siderophores, extracellular enzymes, and antimicrobial metabolites—key determinants of their plant growth-promoting and biocontrol potential[1].

Antimicrobial secondary metabolites constitute a chemically and structurally diverse class of low-molecular-weight compounds capable of suppressing the growth or metabolic activity of competing microorganisms, even at trace concentrations. Several strains of *Pseudomonas* are recognized as prolific producers of such bioactive molecules, including 2,4-diacetylphloroglucinol (2,4-DAPG), phenazines, pyoluteorin, pyrrolnitrin, and hydrogen cyanide, which collectively contribute to biological control activity and improved plant health (Table 1; Fig. 1). Among these metabolites, 2,4-DAPG is particularly notable for its broad-spectrum antimicrobial, antifungal, and nematicidal properties, playing a crucial role in the suppression of soil-borne phytopathogens such as *Gaeumannomyces graminis* var. *tritici*, *Rhizoctonia solani*, and *Pythium ultimum*. The biosynthesis of 2,4-DAPG is governed by the *phl* gene cluster, a conserved genomic locus spanning approximately 8.2 kb and comprising eight contiguous open reading frames (*phlHGFACBDE*)

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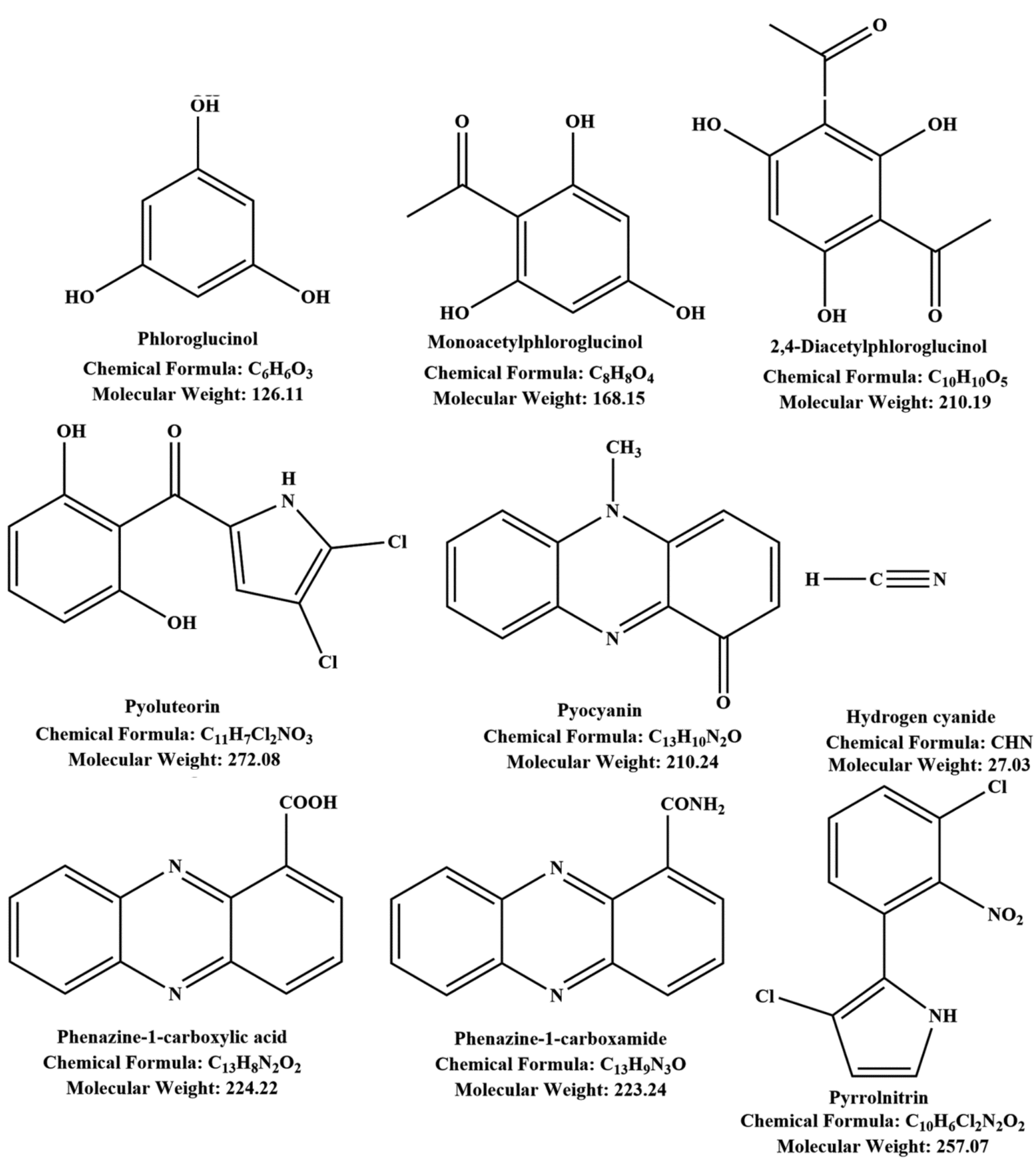

Fig. 1 — List of antibiotics produced by fluorescent pseudomonads.

encoding key enzymes and regulatory components responsible for its production, regulation, and metabolic turnover[2]. Recent comparative genomic analyses have revealed that the *phl* gene cluster is highly conserved across *Pseudomonas protegens*, *P. fluorescens*, and *P. brassicacearum*, yet exhibits genomic rearrangements reflecting evolutionary adaptation to diverse ecological niches[3]. The biosynthesis of 2,4-DAPG is regulated by a multilayered control network involving the GacS/GacA two-component system and the small regulatory RNAs RsmX, RsmY, and RsmZ. This regulatory cascade modulates *phl* gene expression in response to environmental, nutritional,

Table 1 — Antimicrobial metabolites produced by fluorescent pseudomonads

S. No	Bioactive metabolite	Producer strain	References
		Phenazines	
1	Phenazine-1-carboxylic acid	*P. fluorescens* 2-79 *P. aureofaciens* 30-84 *Pseudomonas* sp. M18G *P. fluorescens* Psd	6–9
2	Dimer of Phenazine-1-carboxylic acid	*P. fluorescens* Pf 23	10
3	Penazine-1-carboxamide	*P. aeruginosa* PUPa3 *P. aeruginosa* MML2212 *P. chlororaphis* PCL 1391 *P. aeruginosa* PAO1	11–15
4	2-hydroxyphenazine carboxylic acid	*P. fluorescens* 2-79RN$_{10}$ *P. fluorescens* PGS12 *Pseudomonas* sp. In-b-109 *P. aeruginosa* PNA1	16–19
5	Pyocyanin	*P. aeruginosa*	20
		Phenolics	
6	2,4-diacetylphloroglucinol	*P. fluorescens* Q2-87 *P. fluorescens* CHA0 *P. fluorescens* F113 *P. fluorescens* PFM2 *P. fluorescens* Pf-5 *P. fluorescens* Q8r1-96 *P. fluorescens* S272	21–27
		Pyroles	
7	Pyrrolnitrin	*P. cepacia* B37w *P. fluorescens* *P. fluorescens* BL 915 *P. fluorescens* Psd *P. chlororaphis* O6	28–32
8	Pyoluteorin	*P. fluorescens* Pf-5 *P. fluorescens* CHA0 *P. fluorescens* S272 *P. fluorescens* *Pseudomonas* sp. M18	27,33–36
		Volatiles	
9	Hydrogen cyanide	*P. fluorescens* *P. fluorescens* P.f-68, 100 *P. fluorescens* RAF 15	37–39

and population-density signals, ensuring context-dependent production of the metabolite in the rhizosphere. Recent studies have linked redox regulators (GrxD) and central-carbon metabolism (via *gltA*, encoding citrate synthase) with 2,4-DAPG biosynthetic efficiency, revealing intricate links between primary metabolism and secondary metabolite production. Furthermore, synergistic interactions between 2,4-DAPG and other antibiotics such as pyoluteorin and phenazines enhance the antagonistic potential of these bacteria, improving their persistence and ecological competitiveness in the rhizosphere. Beyond its direct antimicrobial activity, 2,4-DAPG functions as an elicitor of induced systemic resistance (ISR) in host plants by activating jasmonate- and ethylene-mediated signalling pathways, thereby enhancing stress tolerance and providing durable suppression of pathogen attack[4]. Recent advances in multi-omics and genome-scale modelling have provided new insights into the evolutionary conservation, regulatory dynamics, and metabolic optimisation of the 2,4-DAPG pathway, reinforcing its significance as a cornerstone of sustainable crop protection and soil health management[5].

Phloroglucinols are key phenolic secondary metabolites synthesised by diverse biological sources, including microorganisms, plants, and marine organisms. These compounds share a benzene-1,3,5-triol (phloroglucinol) backbone and form a structurally diverse class comprising nearly 60 natural and semi-synthetic derivatives[40]. Representative examples include 2,4-DAPG from *Pseudomonas fluorescens* and *P. protegens*; hyperforin and adhyperforin from *Hypericum perforatum*; flavaspidic acid from *Dryopteris* ferns; trimethylphloroglucinol and phloracetophenone from *Eucalyptus* species; and eckol, dieckol, and phlorofucofuroeckol-A from marine brown algae such as *Ecklonia cava* and *Sargassum thunbergii*[41]. Collectively, these derivatives exhibit broad biological activities, including broad-spectrum antimicrobial, antiviral, antioxidant, anti-inflammatory, anticancer, antihelminthic, insect and mammal antifeedant, cytotoxic and antiherpetic, and plant growth regulating activities[42–45]. The shared phloroglucinol backbone confers redox-reactive and membrane-active characteristics, explaining their multifaceted bioactivity, including the dual biocontrol and therapeutic potential of 2,4-DAPG. Phloroglucinol-based compounds play an important role in activating plant defence mechanisms by inducing the synthesis of phytoalexins and other defence-associated metabolites, thereby strengthening resistance against invading pathogens. Among the phenolic secondary metabolites produced by *Pseudomonas* species, phloroglucinol (PG), monoacetylphloroglucinol (MAPG), and 2,4-DAPG represent the most biologically significant derivatives. Fluorescent *Pseudomonas* strains, including *P. fluorescens* Pf-5, CHA0, and Q2-87, are widely recognized as efficient natural producers of 2,4-DAPG[46].

The contemporary systematic review provides comprehensive insights into the 2,4-DAPG producing *Pseudomonas* strains, with particular focus on their genomic organization, regulatory architecture, biosynthetic pathways, and multifunctional biological activities. Well-characterized strains such as *Pseudomonas fluorescens* Pf-5, CHA0, and Q2-87 are recognized as major natural producers of 2,4-DAPG, highlighting their significance in sustainable crop protection and plant growth promotion strategies. A detailed understanding of 2,4-DAPG, especially its broad-spectrum antimicrobial, antioxidant, anti-inflammatory, and cytotoxic properties, underscores its pivotal role in shaping rhizosphere ecology, enhancing plant health, and opening new avenues for pharmaceutical and biotechnological applications

Methodology

This review is based on experimental studies, critical reports, and peer-reviewed articles that collectively describe the biosynthesis and bioactive potential of 2,4-DAPG. Relevant literature describing the biosynthesis, regulatory mechanisms, and multifunctional biological activities of 2,4-DAPG was systematically reviewed and critically consolidated to highlight its significance in microbial biocontrol and therapeutic applications. A targeted search was performed across major databases, including Web of Science, PubMed, Wiley Online Library, ScienceDirect, and Google Scholar, using specific keywords such as 2,4-diacetylphloroglucinol, *Pseudomonas fluorescens*, biocontrol metabolite, secondary metabolism, antimicrobial mechanism, and anticancer activity. Priority was given to studies providing detailed mechanistic insights, experimental evidence, and applied biotechnological perspectives to develop a comprehensive understanding of the biological and therapeutic significance of 2,4-DAPG.

Isolation and characterisation of 2,4-DAPG

The standardised protocols have been developed for the isolation and characterisation of 2,4-DAPG from fluorescent *Pseudomonas* strains. High-yielding producers such as *P. fluorescens* Q2-87, *P. protegens* Pf-5, and *P. fluorescens* CHA0 are typically cultured in King's B (KB) or Luria–Bertani (LB) media at 28–30°C, pH 6.8–7.2, under aerated shaking (180–220 rpm) for 48–96 h[15,23]. In optimised fermenter conditions, a dissolved oxygen (DO) level of $\geq$30% and a C:N ratio of 10–20 promote higher DAPG yields. After incubation, the culture broths were centrifuged at 10,000 × g for 10 min and filtered through a 0.22 μm membrane. The filtrate is acidified to pH 2.0–2.5 using HCl and extracted two to three times with ethyl acetate (1:1, v/v)[23,27]. The pooled organic phase is dried over anhydrous sodium sulfate and concentrated under reduced pressure at temperatures not exceeding 40°C. The resultant crude extract is subsequently purified by silica gel column chromatography using a hexane–ethyl acetate gradient or by reverse-phase HPLC on a C18 column with an acetonitrile–water mobile phase to obtain purified 2,4-DAPG. Structural elucidation is performed by HPLC (λmax = 270–280 nm), LC–MS ($[M–H]^-$ = m/z 209), ^1H and ^{13}C NMR (aromatic protons at 6.0–6.5 ppm; carbonyl carbons at 196–200 ppm), and FT-IR (O–H stretch = 3200–3500 cm^{-1}; C=O = 1660–1700 cm^{-1}). Quantification of 2,4-DAPG in rhizosphere samples is typically achieved by LC–MS/MS, yielding 0.5–3 ng per 10^5 CFU per g of root surface[22]. These standardised cultivation, extraction, and analytical conditions have enhanced reproducibility and facilitated accurate identification of 2,4-DAPG across diverse *Pseudomonas* strains, supporting its large-scale application in biocontrol, ecological studies, and pharmacological development.

Biochemical and genetic regulation in the biosynthesis of 2,4-DAPG

Polyketide biosynthesis generally proceeds through decarboxylative condensation, utilizing monomer units such as acetyl-CoA, propionyl-CoA, malonyl-CoA, and methylmalonyl-CoA as the fundamental building blocks. These precursors are assembled by the enzyme family known as polyketide synthases (PKSs). PKSs are large, multi-domain enzymes that catalyse the successive addition of these monomers, facilitating the formation of the polyketide backbone. During this process, the monomers undergo decarboxylation and condensation reactions, resulting in the formation of carbon-carbon bonds, which ultimately lead to the production of the polyketide structure. The diversity of polyketide structures arises from the versatility of the PKS enzymes, which can vary in their domain organisation and the specific catalytic mechanisms they employ. This structural variation is further expanded by the potential for tailoring reactions, where additional enzymes modify the core structure, adding functional groups or altering the stereochemistry of the molecule. During condensation, a series of reactions governed by the PKSs leads to the formation of building blocks that are linked via carbon-carbon bonds. PKSs, isolated from bacteria, possess catalytic properties for aromatic compound biosynthesis and are classified into three main types[47].

The biosynthesis of 2,4-diacetylphloroglucinol (2,4-DAPG) is initiated by a type III polyketide synthase that catalyzes the sequential condensation of one malonyl-CoA unit with three acetyl-CoA units to form monoacetylphloroglucinol (MAPG), which is subsequently acetylated to yield 2,4-DAPG. This pathway was first outlined by Dwivedi and Johri[48]. The production of 2,4-DAPG is tightly regulated by a network of global regulators that control the activity of the genes involved in its synthesis. Four key regulators—the sensor kinase GacS, its cognate response regulator GacA, the primary sigma factor RpoD, and the stress-responsive sigma factor RpoS. These regulators collectively coordinate the transcriptional control of the *phl* biosynthetic gene cluster in response to environmental and physiological signals[49]. Notably, amplification of the *rpoD* gene in *P. fluorescens* CHA0, or mutations affecting the activity of RpoS, has been associated with enhanced production of 2,4-DAPG, highlighting the critical role of sigma factor–mediated regulation in optimizing metabolite biosynthesis. The over expression or modification of these sigma factors, which are essential for the transcription of various stress-response and housekeeping genes, has been linked to an increased output of 2,4-DAPG in this bacterial strain. Thus, the production of 2,4-DAPG is not only a function of the enzymatic steps involved in its biosynthesis but is also regulated by complex genetic and environmental factors, allowing *P. fluorescens* to adapt its metabolic processes in response to various stimuli[49].

Bangera and Thomashow were pioneers in isolating the genomic cluster responsible for 2,4-DAPG biosynthesis in *Pseudomonas fluorescens* Q2-87 (EMBL accession no. U41818)[50]. Molecular analysis of the *phl* biosynthetic locus revealed that a 7.2 kb genomic region, including a core 6.5 kb fragment, harbours the principal genes responsible for 2,4-DAPG production in *Pseudomonas fluorescens*. Within this cluster, six open reading frames—*phlA*, *phlB*, *phlC*, *phlD*, *phlE*, and *phlF*—have been identified based on sequence organisation and codon usage patterns consistent with *Pseudomonas* species (Fig. 2). The genes *phlA* to *phlE* are co-oriented and likely transcribed as an operon, whereas *phlF* is arranged in the opposite direction, suggesting a regulatory role[51]. Collectively, these genes encode enzymes and regulatory proteins involved in the biosynthesis, regulation, transport, and turnover of 2,4-DAPG. According to Moynihan *et al.*, the *phl* gene cluster spans approximately 8.2 kb and comprises eight sequential ORFs—phlHGFACBDE—that collectively orchestrate the

Fig. 2 — Genetic map of the *phl* gene cluster (6.5 kb) involved in 2,4-DAPG biosynthesis, regulation, and transport in *Pseudomonas fluorescens* Q2-87[50,51].

biosynthesis, regulation, and transport of 2,4-DAPG in *Pseudomonas fluorescens*[2]. The phl operon exhibits a consistent transcriptional orientation, with all genes transcribed in the same direction, while *phlH* and *phlF* are oriented oppositely. This variation in transcriptional orientation indicates a potential regulatory complexity in the expression of these genes[47].

In *Pseudomonas fluorescens* Q2-87, the gene cluster (*phlACBD)*, edged by *phlF* and *phlE* (an efflux protein gene), remains essential for 2,4-DAPG biosynthesis[50]. The synthesis of 2,4-DAPG involves multiple steps, with the *phlACBDE* operon playing an essential role in forming the precursor molecule, monoacetylphloroglucinol (MAPG). MAPG is a key intermediate that is further processed to produce 2,4-DAPG. The precise roles of the individual genes in this process are essential for understanding the biochemical pathway, with *phlA*, *phlB*, and *phlC* contributing to the formation of acetoacetyl-CoA and subsequently facilitating the transformation of MAPG into 2,4-DAPG. Despite understanding the broader function of these genes, the detailed mechanisms and specific contributions of *phlA*, *phlB*, and *phlC* to the pathway remain an area of ongoing research[47]. One of the most extensively studied genes in the cluster is *phlD*. This gene encodes a novel type III polyketide synthase (PKS), which catalyst the formation of MAPG through the condensation of three malonyl-CoA molecules. Type III PKSs are distinctive enzymes known for their role in polyketide biosynthesis. While they are relatively uncommon in prokaryotes, they are abundant in higher plants, where they contribute to the synthesis of various secondary metabolites, including stilbene and naringenin, which are important for plant defence mechanisms. *PhlD* shares notable similarities with plant chalcone synthases, which also belong to the type III PKS family. This similarity reflects the evolutionary conservation of polyketide synthase (PKS) enzymes across diverse life forms, suggesting that although *phlD* is specialized for 2,4-DAPG biosynthesis in *P. fluorescens*, it likely shares catalytic features with plant enzymes involved in producing flavonoids and other secondary metabolites[47]. Together, the *phl* gene cluster and the associated enzymes play a central role in the biosynthesis of 2,4-DAPG, a compound holds important biocontrol potentials against various plant pathogens. Understanding the detailed genetic and biochemical processes involved in its production is crucial for optimising its synthesis, whether in natural bacterial strains or through engineered systems.

Raaijmakers *et al.* designed specific primers and probes to detect the *phlD*$^+$ gene within populations of fluorescent pseudomonads[52]. The *phlE* gene is involved in the production of a red pigment and facilitates the transport of 2,4-DAPG or MAPG extracellularly[48]. The *phlF* gene, which is oppositely oriented, encodes a repressor specific to the biosynthetic pathway, although the exact mechanism of *phlF* regulation remains unclear[49,50]. The *phlF* and *phlH* genes encode transcriptional regulators that control the expression of the *phl* gene cluster, while the *phlG* gene produces hydrolase enzymes responsible for degrading 2,4-DAPG[15]. The intergenic region between *phlA* and *phlF* contains Pho boxes recognised by the phlF protein, which represses phl synthesis[53]. Some *Pseudomonas* species also produce triacetylphloroglucinol (TAPG) alongside 2,4-DAPG, but the genes responsible for acetylating 2,4-DAPG to TAPG are yet to be identified.

Over expression of *phl* genes

Phloroglucinol is a valuable chemical with various applications in the agriculture, chemical, and pharmaceutical sectors. In the leather industry, it is utilised as a low-toxicity smooth muscle relaxant[54]. Phloroglucinol serves as a precursor for many antibiotics, including 2,4-DAPG. However, the fermentative synthesis of phloroglucinol is often limited by suboptimal yields and high production costs. The production of 2,4-DAPG from phloroglucinol is tightly controlled by diverse transcription factors that coordinate the expression of the *phl* gene cluster. The gene *hfq* is one among those identified and reported that inactivation of *hfq* gene leads to the reduced expression of *phlA,* and a decrease in 2,4-DAPG biosynthesis is observed in mutant *P. fluorescens* 2P34. Restoration and overexpression of this transcription factor enhanced its 2,4-DAPG production. The gene *emhR* encodes a protein that is responsible for the export of intracellular toxic compounds to the environment via an active efflux pump. Deletion of *emhR* gene in *P. fluorescens* 2P24 leads to the accumulation of extracellular 2,4-DAPG inside the cell and hampering its productivity[55]. Meanwhile, the native wild-type *Pseudomonas* spp. is not friendly for genetic manipulation because it produces 2,4-DAPG along with different antibiotics like phenazines, phenolics, pyrrolnitrin, pyoluteorin, etc., which will reduce its large-scale production.

The complete *phl* gene cluster sequence for *Pseudomonas fluorescens* Q2-87 is accessible in the

EMBL database (accession No. U41818). Raaijmakers *et al.* and Frapolli *et al.* developed primers for each locus (Table 2)[52,53]. Cloning and heterologous expression of *phl* locus in recombinant *E. coli* with regulated genetic mechanism and optimising its various biotic and abiotic factors under fermentor-controlled conditions reached its maximum production up to 780 mg/L[56]. Cao *et al.* cloned the coding regions of *phlD* (type III polyketide synthase), *marA* (multiple antibiotic resistance), and *ACCase* (acetyl-CoA carboxylase) in the expression vector pET30a and pACYC deut-1 under T7 promoter and developed a recombinant *E.* coli[57]. Finally, the overexpression of the recombinant strain attains a maximum production of phloroglucinol up to 3.8 g/L. Dong *et al.* identified the monothiol glutaredoxin GrxD as a novel controller of 2,4-DAPG synthesis in *P. fluorescens* 2P24, where mutation of the *grxD* gene, especially alterations to the active-site CGFS motif, notably decreased 2,4-DAPG levels. GrxD-mediated regulation depended on RsmA and RsmE proteins and suppressed *phlF* expression, enhancing 2,4-DAPG production[58]. Loss of *grxD* impaired biocontrol against tomato bacterial wilt, underscoring GrxD's role in 2,4-DAPG synthesis and biocontrol efficacy.

Antimicrobial activity of 2,4-DAPG

The broad-spectrum antimicrobial activity of 2,4-DAPG, produced by fluorescent pseudomonads, has generated significant interest in agriculture due to its effectiveness in suppressing plant pathogens and its vital role in biological control. The metabolite 2,4-DAPG exhibits diverse antifungal, antibacterial, and antihelminthic activities against crop pathogens[50] and displays herbicidal effects similar to 2,4-dichlorophenoxyacetic acid (2,4-D), a widely used post-emergence herbicide for controlling broadleaf weeds in cereals, sugarcane, and plantation crops. Strains of *Pseudomonas fluorescens* that produce 2,4-DAPG, including Pf5, CHA0, F113, Q2-87, and Q8r1-96, have been effectively applied to control root and seedling diseases caused by soil-borne pathogens in various crop plants[23,25,59–61]. These diseases include cotton damping-off caused by *Pythium ultimum*; sugar beet and cucumber damping-off due to *P. ultimum* and *Rhizoctonia solani*; wheat blotch caused by *Septoria tritici*; wheat root rot by *Fusarium oxysporum*; tobacco black rot caused by *Thielaviopsis basicola*; wheat take-all caused by *Gaeumannomyces graminis* var. *tritici*; tomato bacterial wilt by *Ralstonia solanacearum*; rice bacterial blight by *Xanthomonas oryzae* pv. oryzae; rice blast caused by *Magnaporthe grisea*; rice sheath blight by *R. solani*; sheath rot by *Sarocladium oryzae*; and potato cyst nematodes caused by *Globodera rostochiensis* (Table 3). Earlier investigations have shown that fluorescent pseudomonads associated with the banana rhizosphere synthesize 2,4-DAPG, which plays a significant role in their antagonistic activity against root-necrotizing fungal pathogens[62].

The antibacterial activity of 2,4-DAPG is also reported against human pathogens such as *Micrococcus luteus*, *Staphylococcus aureus*, and *S. lividans*[22]. It also exhibits inhibition against vancomycin-resistant *Staphylococcus aureus* (VRSA), *Enterococcus faecium* (VREF), and methicillin-resistant *Staphylococcus aureus* (MRSA)[70–72], which cause nosocomial infection in immunosuppressed patients and plant pathogens such as *Pseudomonas syringae* pv. *tabaci* and *P. syringae* pv. *phaseolicola* (Table 4).

Table 2 — Genes involved in the biosynthesis of 2,4-DAPG with their functions and primers

S. No	Gene locus	Size (bp)	Function of gene	Primer	Sequence	Annealing temperature (°C)
1	*Phl A*	1415	Acetyl transferase	Phl A (F), Phl A (R)	5’-TTGAACTCGTACTTGAT-3' 5’-GGTATAGGATTCCCTCG-3’	45
2	*Phl B*	669	Phl biosynthesis	Phl Bf1, Phl Br1	5’-GGCGGTTACGCGGCGCA-3' 5’-GCAAAGTGTAGACATGA-3’	50
3	*Phl C*	589	Phl biosynthesis	Phl Cf1, Phl Cr3	5’-ATGATCGTCGAGTCCGCCTA-3' 5’-AGGTGTTCGGCCACCAGGAT-3’	50
4	*Phl D*	745	Polyketide synthase	Phl 2a Phl 2b	5’-GAGGACGTCGAAGACCACCA-3' 5’-ACCGCAGCATCGTGTATGAG-3'	67
5	*Phl E*	873	Exporter of DAPG metabolite intermediates	Phl Ef1 Phl Er1	5’-TGTTCCCGGTGATCATGC-3' 5’-ATGCAGATCAGGCTGAA-3'	50
6	*Phl F*	781	Pathway specific repressor	Phl F for Phl F rev	5’-TTCGAGGGAATCCTATACC-3' 5’-GAGTTCAATCATCTGGCCA-3'	55

Table 3 — List of antifungal compound 2,4-DAPG producing strains and their biocontrol ability against phytopathogenic fungi

S. No	Producer strain	Target pathogen	Host	References
1	*P. fluorescens* CHA0	P.u	Tobacco	22
2	*P. fluorescens* CHA0	G.gt	Wheat	23
3	*P. fluorescens* F113	P.u	Sugar beet	26
4	*P. fluorescens* F113	P.u	Sugar beet	63
5	*P. fluorescens* Q2-87	G.gt	Wheat	60
6	*P. fluorescens* Q8r1-96	G.gt	Wheat	61
7	*P. fluorescens* 2P24	G.gt	Wheat	64
8	*P. fluorescens* CHA0	T.b	Tobacco	65
9	*P. fluorescens* Pf-5	P.u, R.s	Cotton, Rice	66
10	*P. fluorescens* 2112	P.c	Red-pepper	67
11	*P. putida* In-b-1821	R.s, S.o, G.gt	Rice	18
12	*P. fluorescens* PFM2	S.t	Wheat	24
13	*P. fluorescens* CHA0	T.b	Tobacco	22
14	*P. fluorescens* P2	M.g, S.o,	Rice	68
15	*P. fluorescens* P7	M.g, S.o, R.s	Rice	68
16	*P. aeruginosa* P10	M.g, S.o, R.s	Rice	68
17	*P. fluorescens* F113	G.r	Potato	59
18	*Pseudomonas* sp. K94.91, P96.25	P.u	Cucumber	69
19	*Pseudomonas* sp. F96.27, P97.1, P97.27, P97.6, P97.26	F.o	Tomato	69

G.gt, *Gaeumannomyces graminis* var. *tritici*; P.u, *Pythium ultimum*; R.s, *Rhizoctonia solani*; T.b, *Thielaviopsis basicola*; P.c, *Phytophthora capsici*; S.t, *Septoria tritici*; M.g, *Magnaporthe grisea*; S.o, *Sarocladium oryzae*; G.r, *Globodera rostochiensis*; F.o, *Fusarium oxysporum*

Table 4 — Antibacterial activity of 2,4-DAPG

S. No	Test bacterium	Disease	Host
1	*Micrococcus luteus*	Skin infection	Human
2	*Pseudomonas syringae* pv. *tabaci*	Wild fire	Tobacco
3	*Pseudomonas syringae* pv. *phaseolicola*	Halo blight	Bean
4	*Staphylococcus aureus*	Scaled skin syndrome	Human
5	Vancomycin resistant *Staphylococcus aureus* (VRSA)	Nosocomial infection	Human
6	Vancomycin resistant *Enterococcus faecium*	Bloodstream infections	Human
7	Methicillin resistant *Staphylococcus aureus* (MRSA)	Nosocomial infection	Human

Role and mechanism of action of 2,4-DAPG in biocontrol activity of soil-borne diseases

The natural biocontrol of soil-borne plant pathogens in agricultural fields often relies on the presence of specific antagonistic microorganisms in the crop rhizosphere or is enhanced through crop rotation practices. Since the 1970s, research has shown that antagonistic *Pseudomonas* species play a vital role in agriculture, primarily through producing antifungal compounds like 2,4-DAPG and phenazine-1-carboxylic acid (PCA). These metabolites help suppress major plant diseases such as wheat take-all decline, tomato Fusarium wilt, potato scab, apple replant disease, and various root and seedling infections. The polyketide metabolite 2,4-DAPG is a vital element of soil biocontrol efficacy and is particularly active in managing take-all disease (TAD) of wheat affected by *Gaeumannomyces graminis* var. *tritici*. The severity of TAD increases under monoculture conditions of wheat or barley but decreases in non-irrigated or dryland systems, where root and crown rots elicited by *Rhizoctonia solani* AG-8, *R. oryzae*, *Fusarium culmorum*, and *F. pseudograminearum* predominate. Indigenous phenazine-producing *Pseudomonas* spp. have shown significant inhibitory effects which exhibit a promising role in the biocontrol of *Rhizoctonia* root rot in wheat[73].

2,4-DAPG exerts strong antimicrobial activity through multiple cellular disruptions. It damages the cell membrane and wall integrity, increasing permeability and causing leakage of cytoplasmic constituents. The compound also interferes with mitochondrial respiration and inhibits ATP synthesis, leading to energy depletion and cell death. Additionally, 2,4-DAPG induces reactive oxygen species (ROS) accumulation, causing oxidative damage to lipids, proteins, and nucleic acids[74,75]. In fungal pathogens such as *Fusarium oxysporum*, *Pythium ultimum*, and *Rhizoctonia solani*, 2,4-DAPG suppresses spore germination and hyphal elongation, while at sublethal concentrations, it acts as a fungistatic agent that modulates enzyme activity and cell signalling. In the rhizosphere, 2,4-DAPG not only suppresses pathogens directly but also elicits induced systemic resistance (ISR) in host plants by activating jasmonate and ethylene signalling pathways and stimulating phytoalexin synthesis, thereby strengthening the plant's innate defence capacity[22].

The population density of 2,4-DAPG–producing *Pseudomonas* spp. in the rhizosphere is a key factor governing the effective suppression of take-all disease in wheat. Notably, the minimum population threshold required for successful biocontrol differs among various 2,4-DAPG–producing strains, reflecting strain-specific variability in their antagonistic potential. For *Pseudomonas fluorescens* strains Q2-87 and Q8r1-96, effective suppression is achieved when bacterial populations reach approximately 10^5 CFU per gram of wheat root or 10^2–10^4 CFU per gram of rhizosphere soil (equivalent to per wheat seed). In contrast, typical phlD$^+$ isolates require higher densities-about 5×10^5 to 2×10^6 CFU per gram of root—to suppress the disease effectively. Populations below these thresholds generally fail to establish sufficient colonisation and are rarely detected on roots in field soils conducive to take-all infection. These findings highlight that maintaining adequate rhizosphere colonisation by 2,4-DAPG-producing *Pseudomonas* is indispensable for consistent biocontrol activity under field conditions[60]. It is estimated that 2,4-DAPG producers constitute about 0.1–1 % of total rhizosphere bacterial populations and occupy roughly 0.1% of the crop root surface area. The production of 2,4-DAPG by rhizosphere bacteria ranges from 0.5–3 ng per 10^5 CFU/gram of root surface[76]. Although *in vitro* inhibitory concentrations of 2,4-DAPG are often higher than those detected *in vivo*, disease suppression in natural soils occurs via combined antibiotic and plant defence mechanisms, as well as sub-inhibitory physiological effects on target organisms, such as interference with quorum sensing and signal blocking[77].

Anticancer and antiviral mechanism of 2,4-DAPG

2,4-Diacetylphloroglucinol (2,4-DAPG) exhibits potent anticancer, antimetastatic, and antiviral properties supported by recent experimental and computational studies. It exhibits strong cytotoxic effects against various human cancer cell lines, including colon (HCT-15, HCT116), lung (A549), breast (MDA-MB-231), and cervical (HeLa) carcinoma cells[78]. The compound induces apoptosis via both intrinsic and extrinsic pathways, characterised by mitochondrial dysfunction, cytochrome c release, Bax activation, and caspase-3-mediated cell death, while concurrently downregulating anti-apoptotic proteins such as Bcl-2, VEGF, and MMP-2. It also suppresses key oncogenic signalling cascades, notably the NF-κB, WNT1, and MAPK1 pathways, thereby inhibiting cell proliferation, metastasis, and angiogenesis[79]. In addition to its apoptotic effects, 2,4-DAPG exerts pronounced anti-inflammatory poteintial, evident from the suppression of LPS-stimulated proliferation of macrophages (RAW 264.7), monocytic cells (THP-1), and peripheral blood mononuclear cells (PBMCs). The downregulation of NF-κB plays a central role, leading to reduced secretion of TNF-α, IL-6, IL-1β, and NO, which collectively attenuate tumour-promoting inflammation. Furthermore, molecular docking and *in silico* analyses have shown strong binding affinities of 2,4-DAPG and its dimeric derivatives toward multiple oncogenic and viral targets, highlighting their potential as lead scaffolds for therapeutic development. Notably, 2,4-DAPG also exhibits antiviral potential against a broad range of enveloped DNA and RNA viruses, including herpesviruses, enteroviruses, HIV, and SARS-CoV-2, primarily through the inhibition of viral proteases and polymerases. Collectively, these findings demonstrate that the bioactivity of 2,4-DAPG arises from an integrated mechanism involving redox imbalance induction, modulation of apoptotic signalling, and suppression of pro-tumorigenic and inflammatory mediators, underscoring its significance as a promising natural anticancer and antiviral agent[45,75,80].

Plant response to 2,4-DAPG-producing rhizosphere pseudomonads

Fluorescent *Pseudomonas* spp. are prominent root-colonising rhizobacteria that produce a diverse array of antimicrobial metabolites, contributing significantly to sustainable agriculture through plant disease suppression and growth promotion. In a detailed study, Phillips *et al.* demonstrated that microbial metabolites, including 2,4-DAPG produced by *Pseudomonas fluorescens*, can directly influence root exudation. Using a stable ^{15}N-labelled isotope approach, they found that exposure of alfalfa roots to 200 µM 2,4-DAPG increased the total net efflux of 16 amino acids by approximately 1,600% within 3 hours. Similar stimulation of amino-acid exudation was also observed in maize and wheat. The study concluded that 2,4-DAPG primarily blocks amino-acid uptake, thereby enhancing net efflux from roots and altering nutrient availability in the rhizosphere[81]. Shreds of evidence are available that plant growth-promoting rhizobacteria produce specific metabolites or iron chelators for competition and colonisation at the root surface, which induce the elicitation of defence reactions from the host plant towards a wide range of plant pathogens, including viruses, bacteria, fungi, and oomycetes, and in certain cases also suppresses insect herbivores.

Plants protect themselves from microbial infection by a combination of systemic acquired resistance (SAR) and induced systemic resistance (ISR). SAR is an immune response activated locally following pathogen invasion, leading to a systemic induction of defence proteins, including pathogenesis-related (PR) proteins. This process is characterised by an early surge in endogenous salicylic acid (SA) levels, which mediates the activation of resistance mechanisms throughout the plant. SAR provides long-lasting, broad-spectrum protection against subsequent infections by diverse pathogens[82]. ISR refers to a stimulated defensive mechanism developed by a plant upon subsequent inoculation with a pathogen and activation of latent innate immune responses. It occurs naturally through the colonisation of the soil-borne microorganisms in the root surface, such as plant-growth-promoting rhizobacteria and mycorrhizal fungi. Microbe-associated molecular patterns (MAMPs) are recognised by plants through specific receptors, triggering a moderate yet effective activation of immune responses in systemic tissues. ISR in *Arabidopsis thaliana* required an intact response from the plant hormones jasmonic acid (JA), ethylene and independently of salicylic acid. The 2,4-DAPG–producing strain *Pseudomonas fluorescens* CHA0 stimulates ISR in tomato and *Arabidopsis thaliana* against the root-knot nematode *Meloidogyne javanica* and the downy mildew pathogen *Hyaloperonospora arabidopsis*. A comparable ISR response has also been reported for the 2,4-DAPG–producing strain *P. fluorescens* Q2-87, highlighting the conserved role of DAPG in eliciting plant defence across host systems[83].

Diversity of 2,4-DAPG producing strains

Identifying and characterising the diversity of the gene cluster is essential to understand how the rhizosphere environment influences 2,4-DAPG-producing *Pseudomonas* spp., which aids in selecting strains with high rhizosphere competence and enhanced efficacy for biological control of plant diseases. Molecular techniques play a significant role in characterising the genetic diversity of 2,4-DAPG producing pseudomonads. For biosynthesis of 2,4-DAPG six genes (*phlA, phlB, phlC, phlD, phlE,* and *phlF)* are involved, and they are conserved among 2,4-DAPG producing pseudomonads isolated from soils worldwide, but the *phlD* gene is used as marker for strain diversity and targeted, because it exhibits sequence polymorphism, which is sufficient to identify and its locus conserved among its producers[15,82]. The genetic diversity among 2,4-DAPG producing *Pseudomonas* sp. has been identified by whole-cell repetitive sequence-based PCR (rep-PCR), enterobacterial repetitive intergenic consensus (ERIC-PCR), BOX-PCR, random amplified polymorphic DNA (RAPD) analysis, amplified ribosomal DNA restriction analysis (ARDRA), sequence analysis of biosynthetic locus, allele-specific PCR and denaturing gradient gel electrophoresis (DGGE)[15,84–86,87]. The above-mentioned molecular techniques are used to detect the existence of genotypic diversity in *phlD*$^{+}$ locus among 2,4-DAPG producers. The *phlD*$^{+}$ gene is a marker for genetic diversity and is involved in the synthesis of monoacetylphloroglucinol[15,84]. Researchers analysed the diversity of *phlD*$^{+}$ gene locus in different environments including rhizosphere soil of maize and non-rhizosphere soil, wheat rhizosphere, pea rhizosphere, sugar beet rhizosphere, Potato rhizosphere and tobacco rhizosphere. Identifying the genotypes of *Pseudomonas* spp. isolated from different rhizospheric fields revealed that their *phlD* gene locus revealed its inter-strain diversity. A total of 22 *phlD*-based genotypes have been identified and designated A to T, pfY, and pfZ (Table 5). Fuente *et al.* reported that 2,4-DAPG producing strains isolated from crop rhizosphere soil worldwide, the D genotype (17 out of 22) has been found dominant in wheat. The dominance of a single genotype on wheat rhizosphere soil is due to the monoculture of a particular crop for multiple years[86,87]. 2,4-DAPG producing *Pseudomonas* sp. differ in antibiotic production by host preference, rhizosphere colonisation, geographic distribution, and their genotypes. Pf-5 and CHA0

Table 5 — Genotype classification of 2,4-DAPG producing *Pseudomonas* spp. isolated from the different crop rhizosphere soil

S. No	Strain	Molecular technique	Plant source	References
	Genotype A			
1	Pf 5	rep-PCR (ERIC-BOX), ARDRA	Cotton	86
2	CHA0, PGNR1, PGNR2	PCR (Allele-specific primer)	Tobacco	84,88
3	MVP1-3	PCR (Allele-specific primer)	Pea	88
	Genotype B			
4	Q2-87	Gene sequence	Wheat	51
5	Q1-87, Q2-1	PCR (Allele-specific primer)	Wheat	88
	Genotype C			
6	STAD 384-97, STAD 376-97	PCR (Allele-specific primer)	Wheat	88
7	P2112	PCR (Allele-specific primer)	Red-pepper	88
	Genotype D			
8	L5. 1-96, FTAD1R 34	PCR (Allele-specific primer)	Wheat	88
9	Q8r1-96	rep-PCR (ERIC-BOX), ARDRA	Wheat	86
10	Q8r1-96, Q128-87, W2-4, QT1-5, QT5-2, FTAD1R33, Q2-5, OC4-1, FFL1R9	RFLP, RAPD, BOX-PCR	Wheat	15
11	11-18	RFLP, BOX-PCR	Maize	89
12	MVP1-6	PCR (Allele-specific primer)	Pea	88
	Genotype E			
13	Q2-2, Q37-87	PCR (Allele-specific primer)	Wheat	88
14	QT6-1, QT3-2, QT4-2	RFLP, RAPD, BOX-PCR	Wheat	15
15	5MR2	rep PCR (BOX), RFLP, PCR (Allele-specific primer)	Pea	85,88
	Genotype F			
16	JMP6, JMP7	rep PCR (BOX), ARDRA, PCR (Allele-specific primer)	Wheat	86,88
17	JMP12	RFLP, RAPD, BOX-PCR	Wheat	15
	Genotype G			
18	FFL1R18	rep-PCR (BOX), ARDRA	Wheat	86
19	FFL3R39, FFL3R48, FFL3R55	rep PCR (BOX), RFLP	Wheat	85
20	FFL1-R 16, FFL1-R 23	rep-PCR (ERIC-BOX), ARDRA	Wheat	87
21	FFL1-R 24, FFL1-R 25, FFL1-R 26	RFLP, RAPD	Wheat	15,86
	Genotype H			
22	CV1-1	PCR (Allele-specific primer), rep-PCR (ERIC-BOX), ARDRA	Wheat	15,86
23	CV2-4, CV4-3	RFLP, RAPD, BOX-PCR	Wheat	15
	Genotype I			
24	FTAD1R36	rep-PCR (ERIC-BOX), PCR (Allele-specific primer), ARDRA	Wheat	86,88
25	FTAD2R-21, 22, 49, FTAD3R-51, 52, FTAD4R-9, 11	rep PCR (BOX), RFLP	Wheat	87
	Genotype J			
26	FTAD1R36, CC3-1, FFL1R22, CC3-6 FFL1R1-14	BOX-PCR, RAPD, RFLP	Wheat	15
27	FFL1R8	rep PCR (ERIC-BOX), PCR (Allele specific primer), ARDRA	Wheat	86,88
	Genotype K			
28	F113	BOX-PCR, RAPD, RFLP	Sugar beet	15,84
	Genotype L			

(*Contd.*)

Table 5 — Genotype classification of 2,4-DAPG producing *Pseudomonas* spp. isolated from the different crop rhizosphere soil (*Contd.*)

S. No	Strain	Molecular technique	Plant source	References
29	W4-4	rep PCR (ERIC-BOX), PCR (Allele-specific primer), RFLP, RAPD, ARDRA	Wheat	15,86,88
	Genotype M			
30	Phl 1C2, Phl H1	PCR (Allele specific primer), rep PCR (ERIC-BOX), ARDRA	Tomato	86,88
31	D27 B1, D27B4, D27B10	PCR (Allele specific primer), BOX-PCR, RAPD, RFLP	Wheat	15,88
	Genotype N			
32	HT5-1	PCR (Allele specific primer)	Wheat	88
33	HT5-10	rep PCR (ERIC-BOX), RFLP, RAPD, ARDRA	Wheat	15,86
	Genotype O			
34	7MA12, 7MA20	PCR (Allele specific primer)	Pea	85,88
	Genotype P			
35	7MA15, 6WSU4, 4MA6, 6MA17, MVP1-4	PCR (Allele specific primer)	Pea	85,88
36	MVW1-1	PCR (Allele specific primer)	Wheat	85,88
	Genotype Q			
37	MVW4-2, MVW4-3	PCR (Allele specific primer)	Wheat	85,88
	Genotype T			
38	FFL2R4, FFL2R5, FFL2R54, FFL2R55, FFL2R61, FFL3R49	PCR (Allele specific primer)	Wheat	85,88
	Genotype PfY			
39	HR3-a13	RFLP	Wheat	90
	Genotype PfZ			
40	PR3-A52	RFLP	Wheat	90

belong to genotype A and produce pyoluteorin and hydrogen cyanide besides 2,4-DAPG, which is responsible for the take-all decline in the wheat rhizosphere[35]. The D Genotype Q8r1-96 is present in wheat rhizosphere, has strong colonisation, competition properties and produces bacteriocin-like inhibitors, making it a superior biocontrol strain to other closely related genotypes of 2,4-DAPG producers[23].

Factors affecting the production of 2,4-DAPG

Microorganisms optimally grow in nutrient-rich, balanced media to increase cell mass, but when growth is restricted due to the limitation of nutrition, certain microorganisms switch on to the synthesis of secondary metabolism to inhibit the competitive microbes in their environment. Abiotic factors such as temperature, pH, mineral content, dissolved oxygen, osmotic potential, carbon, nitrogen, and phosphate sources, as well as biotic factors like indigenous microflora, the pathogen, plant metabolites, and cell density of producing strain, all play a vital role in the production of secondary metabolites[91].

Biosynthesis of 2,4-DAPG in *Pseudomonas* spp. is regulated by multiple endogenous factors, including quorum sensing via N-acyl homoserine lactones, various sigma factors, and the GacA/GacS two-component regulatory system, composed of the membrane-bound sensor kinase GacS and the cytoplasmic response regulator GacA. GacS recognises specific environmental stimuli and in return, GacA triggers the expression of specific 2, 4-DAPG producing genes. The abiotic factor temperature strongly influences the synthesis of antibiotic 2,4-DAPG, which is optimum at 12°C, but other factors, such as pH and iron concentration, have little or no effect on its production[26]. *Pseudomonas* spp. that biosynthesise 2,4-DAPG also produces MAPG, but the MAPG-to-DAPG conversion ratio varies with nutrient availability in different rhizosphere environments. Plant roots and granite chips are added to the culture medium to mimic the natural conditions and explain the strain needed to form microcolonies to produce a significant amount of 2,4-DAPG[92].

The synthesis of 2,4-DAPG in fluorescent pseudomonads is stirred by fructose, sucrose, mannitol, and ethanol, whereas sorbose and glucose repress its production[26]. Zinc sulfate and ammonium molybdate are also reported for the improved 2,4-DAPG production. The production is inhibited to a large extent by inorganic phosphate. A fungal toxin such as fusaric acid produced by *F. oxysporum* f. sp. *radicis-lycopersici*, foliar infection, and physical stress is found to be an effective repressing agent of 2,4-DAPG production, but the presence of pathogens like *P. ultimum* and *R. solani* is involved in the stimulation of *phl* gene[38]. Instead of organic nitrogen, inorganic nitrogen results in no production of 2,4-DAPG[93]. Heterologous expression of 2,4-DAPG producing strains that produce pyoluteorin, salicylate, or other metabolites is balanced such that one antibiotic represses the synthesis of the other[15,94]. The pathway-specific repressor gene *phlF* is located in the *phl* gene locus and codes a DNA-binding protein that plays a vital role in the production of antibiotics at the transcriptional level. The DNA-binding protein represses 2,4-DAPG production during early cell growth to prevent potential self-toxicity, while also functioning as a key regulator that mediates both the autoinduction of 2,4-DAPG synthesis and its repression by salicylate and pyoluteorin. Mutants of *phlF* were found to be unresponsive to 2,4-DAPG–mediated autoinduction and to repression of *phlA* by another metabolite[49]. Jousset *et al.* reported the reduced expression of *phl* and other antibiotic genes involved in plant defence mechanisms due to volatile metabolites released by bacteriovorus rhizosphere inhabitants such as *Acanthamoeba*[95].

Chemical synthesis of natural antibiotic 2,4-DAPG

The biosynthesis of 2,4-DAPG by fluorescent pseudomonads has drawn significant agricultural interest for its role in suppressing plant pathogens such as take-all decline (TAD) in wheat; however, its natural production is limited by slow biosynthesis rates and low yields, necessitating complex downstream processing involving extraction, column chromatography, and repetitive high-performance liquid chromatography (HPLC) purification. Strategies to augment 2,4-DAPG production include the addition of plant root exudates and genetic manipulation through cloning of the *phl* gene locus to enhance metabolic capacity in pseudomonads[57,92]. However, productivity is still low, and it is not economically feasible to meet the demand of agricultural requirements. Large-scale synthesis of 2,4-DAPG is required to meet the demand for natural antibiotics. Marchand *et al.* and Zakrzewski *et al.* reported the simple chemical synthesis procedure and produced a high yield of 2,4-DAPG up to 90%[96,97]. This procedure involves the direct alkylation of commercially available 2-acetylphloroglucinol using acetic anhydride as the acetylation reagent, with boron trifluoride-etherate as a catalyst (Fig. 3). The convenient chemical synthesis of 2,4-DAPG is an alternative to natural antibiotics and reduces the risk of using synthetic pesticides, which are hazardous to our environment.

Discussion

2,4-Diacetylphloroglucinol (2,4-DAPG) is increasingly recognised as a metabolite of dual significance in agriculture and human health. In the agricultural sector, 2,4-DAPG functions as a potent biocontrol agent produced by fluorescent *Pseudomonas* spp., effectively suppressing a broad range of phytopathogens such as *Rhizoctonia solani*, *Pythium ultimum*, and *Fusarium oxysporum*. Its multifaceted mechanism—encompassing inhibition of hyphal growth, disruption of membrane integrity, and induction of oxidative stress—contributes to both direct pathogen suppression and indirect enhancement of plant immunity. Moreover, the metabolite's ability to elicit ISR through jasmonate and ethylene signalling pathways offers a sustainable alternative to chemical pesticides in crop protection systems[98]. By reducing dependence on synthetic agrochemicals, 2,4-DAPG aligns with SDG 2 (Zero Hunger) and SDG 12 (Responsible Consumption and Production) by promoting environmentally sustainable crop protection, enhancing soil health, and contributing to resilient agricultural systems that strengthen long-term food security (Fig. 4).

Recent advances have further revealed that 2,4-DAPG exhibits diverse pharmacological properties, highlighting its potential beyond agriculture. Studies on natural and semi-synthetic phloroglucinols demonstrate that 2,4-DAPG shares structural motifs responsible for potent antibacterial, antiviral, and

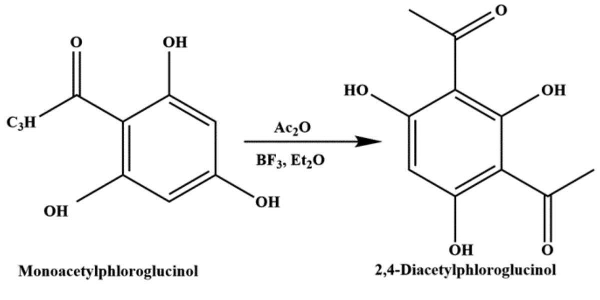

Fig. 3 — Conversion of MAPG to DAPG using acetic anhydride (Ac_2O) and $Et_2O{\cdot}BF_3$ as catalyst[96].

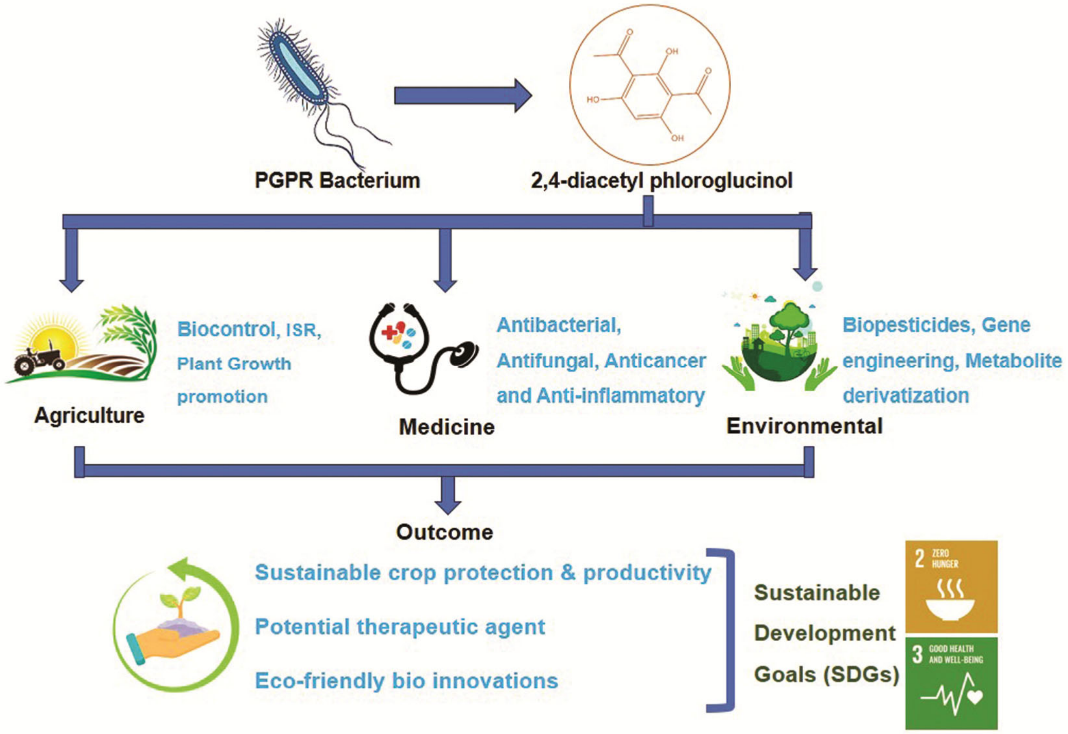

Fig. 4 — 2,4-DAPG: A Multifunctional metabolite bridging agriculture, medicine, and environmental sustainability.

anticancer activities[45]. Experimental evidence suggests that 2,4-DAPG induces apoptosis, inhibits tumour cell migration, and interferes with key signalling cascades involved in inflammation and oxidative stress regulation[99]. These biological effects underline its versatility as a bioactive molecule with implications for both plant protection and human health. In essence, 2,4-DAPG embodies a rare dual functionality: a metabolite vital for rhizosphere ecology and plant defence, yet equally promising as a template for next-generation antimicrobial and anticancer agents. The continued exploration of its biosynthetic regulation, stability, and molecular targets may enable its transition from an agricultural biocontrol molecule to a versatile bioactive agent of therapeutic importance.

Conclusion and future perspective

2,4-Diacetylphloroglucinol (2,4-DAPG) is a key secondary metabolite predominantly produced by fluorescent *Pseudomonas* species and is recognized as a major contributor to the biological suppression of soil-borne plant pathogens. Owing to its wide range of bioactivities—including antifungal, antibacterial, nematicidal, phytotoxic, and cytotoxic properties—2,4-DAPG functions as a central molecular determinant governing plant–microbe and microbe–microbe interactions within the rhizosphere ecosystem. Its mode of action involves disruption of essential microbial processes, including oxidative phosphorylation, RNA transcription, protein synthesis, and induction of oxidative stress, thereby compromising pathogen viability and virulence. Recent advances in molecular and functional genomics have expanded our understanding of 2,4-DAPG biosynthesis and regulation. The *phl* gene cluster (*phlHGFACBDE*) governs its biosynthetic pathway, while regulatory networks such as GacS/GacA–RsmXY/Z, GrxD-mediated redox control, and central carbon metabolism (via *gltA*) modulate production efficiency in response to environmental cues. These findings link primary metabolism and redox homeostasis to secondary metabolite output, providing new avenues for metabolic optimisation. Beyond its antimicrobial role, 2,4-DAPG elicits induced systemic resistance (ISR) in plants by activating the jasmonate and ethylene signalling pathways, contributing to sustained disease resistance and growth promotion. The integration of multi-omics data and genome-scale metabolic models has further refined our understanding of strain diversity, regulation, and ecological adaptation among 2,4-DAPG producers.

In the future, research on 2,4-DAPG should focus on genome-guided discovery and engineering of novel producer strains with enhanced rhizosphere competence and metabolite stability. Employing synthetic biology and metabolic engineering approaches can further improve pathway flux, yield, and formulation consistency. Systems-level modelling of environmental and nutritional parameters influencing *phl* gene expression and metabolite turnover will be vital to predict and enhance production under field conditions. Moreover, 2,4-DAPG holds immense promise beyond agriculture—its emerging anticancer, antimicrobial, and antioxidant properties warrant exploration in biomedical contexts. The development of eco-sustainable bioformulations integrating 2,4-DAPG-producing *Pseudomonas* strains with other beneficial microbes could provide effective, environmentally safe alternatives to chemical pesticides. Collectively, these advancements position 2,4-diacetylphloroglucinol as both a cornerstone metabolite in sustainable crop protection and a potential lead compound in biotechnology and therapeutics.

Conflict of interest

The authors declare that they have no conflicts of interest.

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