Review

Fluopsin C: A Review of the Antimicrobial Activity against Phytopathogens

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Abstract: Fluopsin C (FlpC) is an organocupric secondary metabolite with low-molecular-weight, produced by some \textit{Pseudomonas} and \textit{Streptomyces} bacteria. The compound was identified in 1970 as prismatic dark-green crystals, with strong antimicrobial activity against several human and phytopathogens. Due to its high cytotoxicity, research on this compound decreased after the 1970s. During the early 2000s, FlpC gained more attention as a promising compound by which to develop new antimicrobials to control human, animal, and plant pathogens. This study provides an overview of the results pertaining to the in vitro and in vivo antimicrobial activity of pure FlpC, as well as semi-purified fractions containing FlpC, against phytopathogenic microorganisms. Furthermore, the bioprospection history of the extensively researched FlpC-producing \textit{Pseudomonas aeruginosa} LV strain and the related molecular evidence regarding the compound’s biosynthesis are discussed.

Keywords: natural products; secondary metabolite; \textit{Pseudomonas aeruginosa} LV strain; thioformin cupric complex; organometallic antibiotic compound

1. Introduction

Plant diseases are one of the main causes of productivity losses in crops, with an estimated impact of one billion dollars per year worldwide. These numbers are attained when the effects of phytopathogens and abiotic stresses are combined [1]. Currently, the use of pesticides is essential for maintaining the yield and quality of different crops. The negative effects of some of these chemicals on human, animal, and plant health have been well described, in addition to their potential to contaminate the soil, surface, and groundwater, affecting non-target vegetation and organisms. However, there is little information regarding the real magnitude of the global negative impacts of their use [2].

Secondary metabolites naturally produced by microorganisms are a major source of bioactive compounds, which are used in many biotechnological products produced in different industrial areas, and especially in new antimicrobial development [3,4]. These bioactive compounds could be a source of new molecules to control microorganisms that affect several crops, avoiding massive losses and presenting a lower environmental impact.

Fluopsin C (FlpC) is a secondary metabolite produced by \textit{Pseudomonas} spp. or \textit{Streptomyces} sp. grown in the presence of copper [5,6]. The compound has shown strong antimicrobial activity on a broad-spectrum of human pathogens [7–10] and phytopathogens [11–15]. Additionally, it has been described as a potential antitumoral agent [16,17].

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The study of FlpC decreased after the 1970s due to its elevated cytotoxicity for clinical purposes [7,10], and has increased again over the last few decades with the rise of new technologies for cytotoxicity attenuation and new applications against plant pathogens. In the present review, both the past and present importance of FlpC, and the potential of this compound as a new antimicrobial product against phytopathogens, are discussed.

2. Fluopsin C

The first report of FlpC was published in 1970, in which it was described as a “dark green antibiotic”, purified from cultures of *Pseudomonas* MCRL 10107, called antibiotic YC 73 [7]. Other research groups called it antibiotic B1 [18,19], and organocopper antibiotic compound (OAC) [14], until the purified molecule was identified as FlpC. A timeline of the studies regarding FlpC is presented in Figure 1. Initially, the interest in FlpC was mainly concentrated on the treatment of human pathogens, focusing on the bioprospecting of FlpC-producing strains, methods of purification, screenings for its antimicrobial activity, cytotoxicity tests, and obtention of less toxic derivatives. This shifted after 2000, with the susceptibility of several plant pathogens determined, once its phytotoxic effects had been determined to be minimal [20–34].

Figure 1. The timeline of studies related to Fluopsin C research.
FlpC \([\text{bis}(N\text{-methylthioformohydroxamate})\text{Cu(II)}]\) is a compound with low-molecular-weight (~243,786 g·mol\(^{-1}\)), produced in the secondary metabolism of a few species of \(\text{Pseudomonas}\) and one \(\text{Streptomyces}\) (Table 1) \([6,7,16]\).

Table 1. List of Fluopsin C-producing bacteria.

| Bacteria                              | Reference                   |
|---------------------------------------|-----------------------------|
| \(\text{Pseudomonas MCRL 10107}\)     | [7]                         |
| \(\text{Pseudomonas fluorescens}\)   | [20]                        |
| \(\text{Pseudomonas fluorescens KY 4032}\) | [21]                   |
| \(\text{Pseudomonas reptilivora}\)  | [18]                        |
| \(\text{Pseudomonas aeruginosa 3120}\) | [25]                   |
| \(\text{Pseudomonas aeruginosa LV}\) | [26]                        |
| \(\text{Pseudomonas sp. AV 246 (DSM 15085)}\) | [11]             |
| \(\text{Pseudomonas sp. AV 249 (DSM 15086)}\) | [11]             |
| \(\text{Pseudomonas jinanensis}\)  | [17]                        |
| \(\text{Pseudomonas aeruginosa PAO1}\) | [34]                   |
| \(\text{Streptomyces 4601}\)        | [16]                        |

The FlpC crystals (Figure 2A) are thin, prismatic, and are dark-green or brownish in color (Table 2) \([7,20]\). The compound is formed during a complexation reaction between cupric ions (Cu\(^{2+}\)) and two molecules of the siderophore thioformin (\(N\text{-methylthioformohydroxamic acid}\)) (Figure 2B), an important component in the transport system of ferric ions (Fe\(^{3+}\)) through the membrane of algae, fungi, and bacteria \([27,35]\).

Figure 2. Fluopsin C. (A) scanning electron microscopy micrography of Fluopsin C crystals; (B) molecular structure of Fluopsin C (\(\text{C}_4\text{H}_8\text{N}_2\text{O}_2\text{S}_2\text{Cu}\)), a coordination compound formed by two molecules of the thiohydroxamic siderophore thioformin (\(\text{C}_2\text{H}_5\text{NOS}\)) bonded with one central metal ion of copper.

Several metallic ions are able to complex with thioformin, forming Fluopsins that present antimicrobial activity \([27]\), as shown in Table 2. These complexes are obtained by microbial biosynthesis or chemical synthesis, including derivative molecules \([23,24,36,37]\).
Table 2. Main organometallic complexes in the Fluopsin group (thioformin complexes).

| Fluopsin | Complexed Ion | Morphology       | Minimum Inhibitory Concentration | Reference |
|----------|---------------|------------------|----------------------------------|-----------|
|          |               |                  | Staphylococcus aureus FDA 209P (µg mL⁻¹) | Bacillus subtilis PCI-219 (µg mL⁻¹) |          |
| C        | Cu²⁺          | Dark-green needles | 0.09                             | 0.09      | [7]       |
| CA       | Ca²⁺          | Colorless needles | 0.39                             | 0.78      |           |
| F        | Fe³⁺          | Black needles     | 0.39                             | 3.12      |           |
| NI       | Ni²⁺          | Dark red needles  | 0.39                             | 0.39      | [22]      |
| Z        | Zn²⁺          | Colorless prisms  | 0.78                             | 1.56      |           |
| S        | Sn²⁺          | Pale brown powder | 0.78                             | 1.56      |           |
| N        | Na²⁺          | Colorless needles | 0.39                             | 0.78      |           |

*S. aureus FDA 209P (µM) Escherichia coli NIHJ (µM)*

|          |               |                  |                               |           |
| PD       | Pd²⁺          | Red crystals     | 0.50                           | 5.00      | [24,35]   |
| PT       | Pt²⁺          | Yellow slurry    | NE * at 150.00                 | NE at 150.00 |           |
| CO       | Co³⁺          | Brown crystals   | 5.00                           | 75.00     |           |
| CR       | Cr³⁺          | Green solids     | 25.00                          | 75.00     |           |
| RH       | Rh³⁺          |                  | 150.00                         | NE at 250.00 |           |

* No effect (NE).

3. Fluopsin C-Producing Microorganisms

Until now, a few bacteria have been reported as FlpC producers, and almost all strains are from the *Pseudomonas* genus, especially the *P. aeruginosa* LV strain, which is the most studied strain.

The *P. aeruginosa* LV strain was isolated from old leaf lesions of orange (*Citrus sinensis* cv. Valencia) with citrus canker disease at Astorga city (Paraná State, Brazil) in 2004 [26]. Recently, its genome has been sequenced (GenBank CP058323) [33].

Curiously, in the orchard where LV strain and other bacterial strains were isolated, the control of citrus canker disease was carried out by spraying copper chloride solution on leaves and fruits. Most likely, the presence of a large amount of copper chloride selected tolerant bacteria strains, co-existing with *Xanthomonas citri* pv. *citri* inside the citrus canker lesions. The mechanisms of the LV strain to protect itself against copper chloride are proposed and are discussed here.

*Molecular Insights on Fluopsin C Biosynthesis*

The process of FlpC production by bacteria has not been completely elucidated, and efforts have been made using molecular biology to identify the genes involved in the biosynthesis pathway.

It is known that the presence of copper (Cu²⁺) in the culture media is a trigger for FlpC production. Transcriptome analysis of the *P. aeruginosa* LV strain, cultivated in the presence and absence of CuCl₂, indicated that nine genes were differentially expressed (hyperexpressed) when compared with bacteria cultivated in the absence of CuCl₂ [5]. The analysis also showed that the genes should be functionally grouped as metallic ions transporters (*PA3574a; PA3920; PA3523; PA3251*); transcriptional regulators (*PA4878*); phenazines biosynthesis (*phzA2*); and hypothetical proteins (*PA2691; PA4141; PA4782*).

Most of the detected hyperexpressed genes were related to metallic ions transport systems, strongly indicating that FlpC biosynthesis occurred as a bacterial bioremediation mechanism, to protect itself against high intracellular levels of copper [5]. The ions cause high cytotoxicity, disrupting iron-sulfur enzyme complexes and generating reactive oxygen species (ROS) [38].
The \textit{phzA2} gene belongs to \textit{phz2 operon}, which is responsible for basal production of phenazine-1-carboxylic acid (PCA). The PCA acts as a signaling molecule, inducing \textit{phz2 operon} transcription and the expression of \textit{phz1 operon}, increasing PCA biosynthesis [39].

Based on the experimental data described above, the current hypothesis considers that PCA should be a metabolic precursor of thioformin. However, it is still unclear which precursors participate in FlpC biosynthesis. The synthesis of FlpC remains under investigation, and future studies comprising genetic silencing will indicate the action of those hyperexpressed genes detected in the FlpC pathway.

On the other hand, in the presence of elevated concentrations of copper, the transcriptome analysis of \textit{P. aeruginosa} PAO1 revealed the hyperexpression of different operons, such as \textit{PA3515-3519} (which encodes five proteins with unknown functions) [40], \textit{copZ1} (which encodes a copper chaperone), and \textit{PA3521-PA3523} (which encodes an efflux pump) [38]. The presence of copper was detected in the cytoplasm by the Cu-sensing transcriptional regulator CueR, which activates these genes to re-establish copper homeostasis [34,38,41].

Patteson et al. [34] investigated if \textit{PA3515-3519 operon} proteins could be responsible for the synthesis of FlpC. By the heterologous expression of the operon in \textit{P. fluorescens} SBW25 cultured with CuSO\textsubscript{4}, it was possible to confirm that it contained all the necessary genes for FlpC production, being named as the \textit{flc} gene cluster (PA3515-3519 as \textit{flcA} to \textit{flcE}).

After cluster determination, \textit{P. aeruginosa} PAO1 was cultured in M9 minimal medium with CuSO\textsubscript{4} and [\textsuperscript{15}N,\textsuperscript{13}C\textsubscript{3}] L-methionine or [\textsuperscript{15}N,\textsuperscript{13}C\textsubscript{5}] L-cysteine, in order to evaluate the incorporation of the amino acids into FlpC, using Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) analysis. The results indicated the incorporation of one carbon from L-Met (possibly for the N-methyl group), and then one carbon and one nitrogen from L-Cys (possibly to form the thiohydroximate backbone) to the antibiotic [34]. These findings enabled the investigation of each reaction involved in FlpC production and the proposition of a biosynthesis pathway model.

In the proposed model by Patteson et al. [34] (Figure 3), the biosynthesis of FlpC starts by FlcB catalyzing the conjugation of L-cysteine with fumarate, forming the precursor 2 (C\textsubscript{7}H\textsubscript{12}NO\textsubscript{6}S\textsuperscript{+}; 238.040 m/z). Then, the heme oxygenase FlcE catalyzes oxidative decarboxylation and N-hydroxylation reactions converting 2 to 3 (C\textsubscript{6}H\textsubscript{10}NO\textsubscript{5}S\textsuperscript{+}; 208.028 m/z). FlcD, another heme oxygenase protein, catalyzes a methylene excision in the 2-C of 3, forming 4 (C\textsubscript{5}H\textsubscript{8}NO\textsubscript{5}S\textsuperscript{+}; 194.012 m/z) and formate. The lyase FlcC catalyzes the cleavage of the C-S bond of 4, forming 5 (thiohydroxamate) and fumarate, the latter being recycled for the first step of the pathway. Finally, FlcA methylates 5, in the presence of SAM (S-adenosyl-L-methionine), generates 6 (Cu-free fluopsin), that binds to the surrounding copper ions. Additionally, the omission of any Flc enzyme fully depletes the production of FlpC.

When comparing genomes using a \textit{Pseudomonas} genome database, the \textit{flc} cluster was present in 99.6% of the 4955 annotated genomes from environmental and clinical isolates; additionally, the cluster was also present in \textit{Acinetobacter baumannii}, \textit{Enterobacter cloacae}, \textit{Streptococcus dysgalactiae} (clinical isolates), \textit{Lysobacter enzymogenes}, and \textit{Lysobacter capsica} (bacteria colonizing roots and leaves) [34].

Different patterns of genetic components were obtained by transcriptomic analysis between LV and PAO1 strains of \textit{P. aeruginosa}, generating two current hypotheses for the synthesis of FlpC. Further studies should allow for the determination of which one is the most accurate, or if the synthesis of the compound comprises different metabolic pathways depending on the bacterial strain.
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Figure 3. Fluopsin C biosynthetic pathway model highlighting the reactions catalyzed by the Flc enzymes. Reprinted with permission from AAAS. Copyright 2021 from ref. [34]. The structural contribution of L−Cys and L−Met are highlighted by the colors red and blue, respectively. Each molecular potential precursor was named as molecules 1-6. SAM: S−adenosyl−L−methionine; SAH: S−adenosyl−L−homocysteine.

4. Fluopsin C In Vitro and In Vivo Inhibitory Effects on Phytopathogens

Fluosins are promising antimicrobial molecules [22], especially FlpC, which controls important clinical and phytopathogenic microorganisms.

Recent studies have used semi-purified fractions containing FlpC, obtained by organic solvent extraction of $P$. aeruginosa LV strain culture supernatant, followed by column chromatography, vacuum liquid chromatography (VLC), and flash chromatography (FC).

Each step generated specific fractions, containing different amounts of FlpC and other compounds. FlpC was found in fractions named as F3 [8,12,13], F3d (30% FlpC) [9,14], and F4A (25% FlpC) [6,15,32].

The use of FlpC against phytopathogens is a more recent approach, and a restricted number of susceptible bacteria, fungi, and oomycetes have been reported to date (Table 3).
**Table 3.** Susceptibility of phytopathogens to Fluopsin C and to semi-purified fractions (F3, F3d, and F4A).

| Microorganisms                        | Minimum Inhibitory Concentration (µg mL\(^{-1}\)) | Reference |
|---------------------------------------|---------------------------------------------------|-----------|
| **Gram-negative**                     |                                                   |           |
| *Pseudomonas syringae* var. *syringae* | 2.00–3.00                                         | [11]      |
| *Xanthomonas citri* pv. *citri*       | 19.53 (F3)                                        | [12]      |
| *Xanthomonas axonopodis* BSC475a       | 100.00 (F3)                                       | [28]      |
| *Xanthomonas arboricola* pv. *pruni*   | 50.00 (F3)                                        | [13]      |
| *Xanthomonas citri* pv. *citri*       | 0.12; 6.25 (F3d)                                  | [14]      |
| *Pectobacterium carotovorum* subsp. *carotovorum* | 7.81 (F4A)                                      | [15]      |
| **Fungi**                             |                                                   |           |
| *Bipolaris sorokiniana*               | 0.40–0.60                                         |           |
| *Fusarium culmorum*                   | 5.00–6.00                                         |           |
| *Fusarium oxysporum*                  | 4.00–5.00                                         | [11]      |
| *Heterobasidion annosum*              | 0.10–0.20                                         |           |
| *Sclerotinia sclerotiorum*            | 6.00–7.00                                         |           |
| **Oomycete**                          |                                                   |           |
| *Pythium ultimum*                     | 0.10–0.20                                         | [11]      |

*X. citri* pv. *citri* is the causal agent of citrus canker disease that affects fruit, leaves, and young stems of citrus plants, promoting lesions that reduce fruit quality and production. Scanning electron microscopy (SEM) analysis of *X. citri* pv. *citri* cultures treated for 1 h with F3 MIC (minimum inhibitory concentration) revealed reduced exopolysaccharides (EPS) content, without cellular disruption. In 3–6 h treatments, the EPS decreased, and cells were shrunken and rough [12].

In a transmission electron microscopy (TEM) examination, after 1 h of treatment with F3, *X. citri* pv. *citri* cells were elongated, and membrane alterations were observed when compared to the control. After 3 h, the cell wall and cytoplasmatic membrane were disrupted, evolving to cell lysis after 6 h.

The same study also reported that, in the orange tree (*C. sinensis* cv. Valencia) infection model, the application of 10 µg·mL\(^{-1}\) of F3 solution reduced leaf lesions by around 93.5% in preventive and curative treatments based on F3 aspersion.

In addition, the F3 fraction showed antibiotic activity against leaf blight disease caused by *Xanthomonas axonopodis* in *Eucalyptus* spp. (MIC of 100 µg·mL\(^{-1}\)), which causes lesions on leaves that evolve necrosis until the leaves fall, reducing the photosynthetic area and plant growth [28]. In SEM, the authors observed that after 1 h of treatment with 200 µg·mL\(^{-1}\) F3d, no EPS was observed in cultures of *X. axonopodis*, and after 3 h, the cells were completely disrupted. The treatment of *Eucalyptus urograndis* 1404 seedlings with 1000 µg·mL\(^{-1}\) of F3, previous to or after pathogen inoculation, reduced foliar lesions by 93.9% and 89.7%, respectively, 30 days after treatment.

Similar studies were carried out using *X. arboricola* pv. *pruni*, one of the major phytopathogens of peach orchards, which causes bacterial spot disease [13]. Cultures treated with 200 µg·mL\(^{-1}\) of F3 showed the same positive effects observed in other studies.

Regarding the reduction of bacterial spot leaf lesions promoted by F3 on peach trees (*Prunus persica* cv. Maciel), no significant differences were found by spraying leaves with 150 or 450 µg·mL\(^{-1}\), as a post- or pre-treatment, and both concentrations reduced the lesions by >70.0%. However, the higher concentrations used in the study promoted phytotoxic effects on peach leaves.

Once it was determined that the F3 fraction was promising against phytopathogens, a new system of purification was developed to achieve a higher resolution in the purification of antimicrobial molecules. Oliveira et al. [14] partitioned F3 into nine new fractions, in which F3d presented increased antimicrobial activity; it was composed by two molecules,
phenazine-1-carboxamide (PCN) and OAC. The OAC was determined later as FlpC, being 30% of F3d mass.

Using F3d, preventive and curative regimens against *X. citri* pv. *citri* were reassessed. In a preventive regimen, 1 µg·mL⁻¹ of F3d led to a lesion-reducing effect of 90.0%, 10 µg·mL⁻¹ reduced 93.0%, and 100 µg·mL⁻¹ reduced 97.0%, with the preventive approach being more effective than curative treatments.

After 120 h of preventive treatment, just a few cells of the phytopathogen were found in the mesophyll, presenting morphological changes, cell lysis, and reduced EPS. Mesophyll cells seemed intact during the entire treatment. Additionally, the activity of the compound was maintained for weeks on the phylloplane and inside the leaves, reducing the potential inoculum and the phytopathogen transmission, without phytotoxic effects.

F4A is a semi-purified fraction obtained by a new system of VLC purification method, which provides a fraction with 25% FlpC (similar to F3d content). The antibiotic activity of the F4A fraction was evaluated against *Pectobacterium carotovorum* subsp. *carotovorum*, and F4A promoted bacterial cell lysis after 3 h of treatment, verified by SEM. In tomato plants (*Solanum lycopersicum* cv. Santa Clara), the preventive regimen provided a survival rate of 100.0% of plants using 7, 70, and 700 µg·mL⁻¹, without displaying any signs of cytotoxicity. In addition, plants preventively treated with 70 µg·mL⁻¹ recorded the highest productivity, both in terms of weight and number of fruits. The curative regimen promoted 20.0% plant survival with 70 and 700 µg·mL⁻¹, and 100.0% with 7 µg·mL⁻¹. Plants treated with lower doses reached the highest growth when compared with other treatments [15].

Pistori et al. [6] investigated the effect of F4A foliar aspersions on the non-axenic culturable *Candidatus Liberibacter asiaticus* (causal agent of Huanglongbing) in *C. sinensis* cv. Valencia infection. Preventive treatments with 100 µg·mL⁻¹ of F4A led to only 14.2% of the citrus trees being positive for pathogen presence after 120 days of bud-grafting inoculation; after 240 days, 57.4% of the trees were positive. Curative treatments at the same concentration demonstrated that 0.0% and 42.8% of the trees were positive for the pathogen after 120 and 240 days, respectively.

The fraction was also indicated as a potential elicitor for the plant systemic acquired resistance (SAR) in orange plants against Huanglongbing, based on the increase of PR-2 gene expression (codifies β-1,3 glucanase). This increase was associated with the presence of the phenazines PCA and PCN in F4A, indicating that the different molecules contained in the semi-purified fraction may synergically act in producing multiple defensive responses against phytopathogens.

More recently, a study dedicated to the investigation of ultrastructural effects promoted by pure FlpC and F4A indicated that they are similar (Table 4) [32]. Additionally, damage to the cytoplasmatic membrane was also assessed by live/dead staining after 15 min exposure to FlpC and F4A, indicating severe damage to the structure by considerably increasing its permeability. The results corroborated the current hypothesis that the main action site of FlpC on Gram-negative and Gram-positive bacteria is the cytoplasmatic membrane, which becomes more permeable [32].

A secondary effect observed in *X. citri* subsp. *citri* 306 was the dissolution of the Z-ring, impairing cell septation and, consequently, the cell division process. This may occur due to the breakdown of the membrane potential, which is essential for positioning FtsZ proteins on the cell division axis. Still, no effects were observed regarding the genetic content or the chromosomal segregation process [32].

Under field conditions, F4A-treated soybeans presented decreased infection by *Phakopsora pachyrhizi* (Asian soybean rust), and when an association of 10 µg·mL⁻¹ F4A plus Sphere Max® (Bayer, São Paulo, Brazil) (a commercial fungicide) was used, the arbuscular mycorrhizal fungi colonization was higher in treated plants (77.84%) compared to other treatments. The nitrogen and phosphorous foliar content increased in treated plants, as well as in terms of seed proteins, plant height, and yield (4.45 ton.ha⁻¹). The combination of F4A (10 µg·mL⁻¹) and Sphere Max® promoted higher fungicide activity on *P. pachyrhizi*,...
with more dead spores and hyphae. However, treatments consisting of F4A alone were unable to control the disease [31].

**Table 4.** Ultrastructural effects in microbial cells exposed to Fluopsin C and F4A fraction analyzed by scanning (SEM) and transmission electron microscopy (TEM).

| Bacterial Model * | Ultrastructural Effects |
|-------------------|-------------------------|
| *Xanthomonas citri subsp. citri* 306 | Disruption of cytoplasmic membrane; shrunken and rough cells. | Modified cellular morphology; elongated cells; indistinction between the cytoplasmic membrane and cell wall. |
| *Klebsiella pneumoniae* KPC19 | Cell wall and membranes disrupted. | Alterations in the cell wall, cytoplasmic membrane, and cytoplasm. |
| *Enterococcus faecalis* ATCC 6569 | Absence of marked differences; reduction in the number of cells. | Absence of marked differences. |
| *Staphylococcus aureus* MRSA N315 | Alterations in the cell wall and cytoplasmic membrane. | Cell wall loss; heterogeneous and electron-dense cytoplasm. |

* Cells suspensions were treated with IC$_{90}$ concentrations of F4A and FlpC [32].

### 5. Conclusions

The development of new antimicrobial agents derived from natural sources is crucial to enhance the control of human, animal, and plant pathogens that are resistant to currently used compounds. In crops, they can also represent new products to control different plant diseases, reducing productivity losses and the negative environmental impacts of chemical pesticides.

FlpC is a promising antimicrobial bio-compound produced by bacteria. However, the number of studies on this molecule is still relatively low, especially considering its cytotoxicity. This is probably the main reason why pioneering research groups neglected investigations on this compound for so long. Research regarding FlpC has reemerged during the last decade, with several studies focusing on the antimicrobial activity of different human and phytopathogens under in vitro and in vivo conditions.

To date, results have indicated that FlpC applications in plant protection are promising and require a low concentration of the compound to be effective. Furthermore, when combined with other secondary metabolites or commercial products, it may synergistically act in promoting multiple beneficial responses.

Still, no attempts have been made to evaluate the environmental changes promoted by the introduction of FlpC in crops. We hypothesize that, due to the molecule’s natural occurrence, which may be frequent in areas where copper solutions are used for phytopathogens management, its biocompatibility is suitable, since these areas operate without reported damage. However, as a broad-spectrum antimicrobial, inappropriate and excessive use of the compound could alter the composition of soil microbiota.

In the future, FlpC should have its environmental impacts determined, especially regarding depletory effects on soil communities, its degradation time and accumulation, its runoff potential towards superficial and underground water, and its effect on non-targeted organisms. Regarding FlpC cytotoxicity, the use in formulations with addition of other compounds could be a useful tool against plant diseases.

There is still a lot of research to be conducted regarding FlpC, but the great potential of this compound is undeniable. In the last few decades, many studies have been conducted, but these only represent a beginning in explorations of the potential of FlpC.
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