Review

Microbial Pyrrolnitrin: Natural Metabolite with Immense Practical Utility

Shraddha Pawar 1, Ambalal Chaudhari 1, Ratna Prabha 2, Renu Shukla 2 and Dhananjaya P. Singh 2,*

1 School of Life Sciences, Kavayitri Bahinabai Chaudhari North Maharashtra University, Jalgaon 425001, India
2 ICAR-National Bureau of Agriculturally Important Microorganisms, Maunath Bhanjan 275101, India
* Correspondence: Dhananjaya.Singh@icar.gov.in

Received: 6 June 2019; Accepted: 12 July 2019; Published: 3 September 2019

Abstract: Pyrrolnitrin (PRN) is a microbial pyrrole halometabolite of immense antimicrobial significance for agricultural, pharmaceutical and industrial implications. The compound and its derivatives have been isolated from rhizospheric fluorescent or non-fluorescent pseudomonads, Serratia and Burkholderia. They are known to confer biological control against a wide range of phytopathogenic fungi, and thus offer strong plant protection prospects against soil and seed-borne phytopathogenic diseases. Although chemical synthesis of PRN has been obtained using different steps, microbial production is still the most useful option for producing this metabolite. In many of the plant-associated isolates of Serratia and Burkholderia, production of PRN is dependent on the quorum-sensing regulation that usually involves N-acylhomoserine lactone (AHL) autoinducer signals. When applied on the organisms as antimicrobial agent, the molecule impedes synthesis of key biomolecules (DNA, RNA and protein), uncouples with oxidative phosphorylation, inhibits mitotic division and hampers several biological mechanisms. With its potential broad-spectrum activities, low phototoxicity, non-toxic nature and specificity for impacts on non-target organisms, the metabolite has emerged as a lead molecule of industrial importance, which has led to developing cost-effective methods for the biosynthesis of PRN using microbial fermentation. Quantum of work narrating focused research efforts in the emergence of this potential microbial metabolite is summarized here to present a consolidated, sequential and updated insight into the chemistry, biology and applicability of this natural molecule.

Keywords: Halometabolites; pyrrolnitrin; biosynthesis; biochemistry; spectral properties; antifungal activity; applications

1. Introduction

Of 5–30 million species on the Earth, fewer than 2 million have been described and fewer than 1% have been explored for a vast repertoire of new natural products with socio-economic significance [1]. Hence, it is reasonable to expect that many more natural products not only from known species, but also from unidentified organisms are yet to come to benefit humanity and the environment [2]. Natural products offer unique structural molecules unparalleled by any other molecular family with an array of biological activities such as for drug leads. The many natural products that occupy the market today without any chemical modification are a testimony to the remarkable properties of secondary molecules produced by an array of plants, insects, animals, microbes and numerous species of marine organisms [3].

Secondary metabolites are small heterogenous organic molecules [4] that display prominent ecological benefits to the host organisms in providing defense against predators, parasites, diseases, interspecies nutritional competence, and competitive edge over interaction with the environment [5,6].
Extensive microbial structural diversification has led to maximizing chemical diversity in terms of the secondary metabolite resources that triggered scope for new drug leads [7]. Since natural products have reflected a wide array of therapeutic and biological applications (antibiotic, anti-inflammatory, antimicrobial, antitumor, anticancer, antiparasitic and immunosuppressing agents as well as enzyme inhibitors), the scope for further exploration of uncharacterized molecules of plant and microbial origin has always remained a focused area for identifying new leads for pharmaceutical and agro-chemical usages [8].

Continuously changing environmental patterns, the emergence of new diseases and resurgence of resistance towards existing drugs have led to an extensive search for novel natural metabolites at a rapid rate [9]. Low molecular size secondary metabolites from living entities have been obtained with typical therapeutic, biological and agricultural implications including antimicrobials, growth promoters, disease suppressors, enzyme inhibitors, health stimulators, and biocontrol agents against pathogenic fungi, bacteria and insects [10,11]. Systematic strategies for obtaining bioactive metabolites include isolation and identification of known secondary metabolites with biological activities unmatched with the molecular libraries or search for unknown natural molecules with versatile bioactivities. For both these options, the microbial world offers a great repository of natural molecules due to their extensive chemical diversity. However, there remains limitations of the culturability of microbial species and the expression of desired molecular traits or chemical species under isolated culture conditions. To overcome this, metagenomics has emerged to represent vast structural diversity of taxonomic communities with multi-functionalities having diverse chemical structures and functions [12].

Thus, secondary metabolites are supposed to be conserved in the species, evolved in a competitive environment, emerged to serve purposes other than primary metabolism, secreted for specific physiological or defense-related reasons, related with the habitat of producing organisms, blessed with complex chemical structures and clubbed with diverse bioactivity [13,14]. These attributes potentiate the usefulness of structurally diverse but functionally sound microbial biomolecules in therapeutics, and agricultural, industrial and environmental applications. We discuss structural, chemical, biological and functional perspectives of one of the earliest known pyrrole antibiotic antifungal metabolite of microbial origin, pyrrolnitrin, which has witnessed laboratory to commercial implications.

### 2. Halometabolites with Potential Functions

Secondary metabolites have emerged as a potential tool against many diseases after 1980 [15]. These molecules account for nearly 67% of the total antibiotics produced [16,17]. Secondary metabolites with halogen moiety in their chemical structure, referred to as “halometabolites”, display wide structural diversity with unique biological functions [18] (Table 1). Earlier, 29 halometabolites were reported with various functions [18] but now more than 5000 natural organohalogens, predominantly chlorinated and brominated compounds, have been identified [19]. These halometabolites are produced by several organisms including microbes, sponges, higher plants and insects. Organisms undergoing abiotic stresses such as extreme conditions, forest fires, volcanoes and volcanic eruptions that lead to abiotic oxidation of organic matter are more prone to halo-compound synthesis [20]. Initially, halometabolites were considered nothing more than an oddity, but later they attracted more attention because of their biogenesis, structural diversity and potential bioactivity.
Table 1. Structural diversity of organohalogen secondary metabolites from various organisms inhabiting different habitats.

| Organohalogens          | Bioactivity                      | Halogen Type and Number | Source, Habitat                        | Reference(s) |
|-------------------------|----------------------------------|-------------------------|----------------------------------------|--------------|
| 4-Chloroindole Ester    | Plant growth promoting hormone   | Cl (01)                 | Pisum Sativum (Lentil, Sweet Pea, Pea, Vetch); Soil | [21,22]      |
| 3-Chloroindole acetate  | Plant hormone                    | Cl (01)                 | Psychodera P. Logania, Marine acorn worm | [23]         |
| Romucosine B            | Plant alkaloids                  | Cl (01)                 | Rollina mucoa; Tropical south America  | [24]         |
| Neotriptrietao          | Diterpene                        | Br (01)                 | Laurencia yonaguniensis; Yonaguni island, Japan | [25]         |
| Bromomethane            | Fumigant, pesticides             | Br (01)                 | Cabbage, Broccoli, Turnips, Rape seed (Family: Brassicaceae); Soil | [26]         |
| 2-Chloro-4-Nitrophenol  | Fungicide                        | Cl (01)                 | Stephanoa Carotolor; Soil               | [26]         |
| Tyrosine derivative     | Improving adhesion between protein fiber, sheets | Cl (01-03)             | Marine Sponges, Sea fans, Gorgonian; Sea water | [27]         |
| Diiodotyrosine          | Precursor in production of thyroid hormone | I (02)                 | Gorgoria Catelli, Sea Fan; Western Atlantic Ocean | [28]         |
| Ecuadororn              | Analgesic activity               | Cl (01)                 | Epppedebates; Eastern Atlantic Ocean   | [29]         |
| Tyrian Purple Dye       | Dye                              | Br (02)                 | Marxes Brandaris; Sea snail            | [30]         |
| Drosophilin A           | Antibiotic                       | Cl (04)                 | Drosophila Substrata; Ligandemetic Basidionyct; Overripe or rotting fruit | [31]         |
| 2,6 Dichlorohphenol     | Sex pheromone, growth hormone    | Cl (02)                 | Female; Penicillium Mold; Decaying material | [26]         |
| 2,4 Dichlorophenol      | Broad spectrum herbicides        | Cl (02)                 | Penicillium Spp.: Agricultural inoculant | [26]         |
| Epibatidine             | Pain killer                      | Cl (01)                 | Epppedebates Anthrope (Frog); Central; Southern cuador | [32]         |
| Chloramphenicol         | Antibiotic                       | Cl (02)                 | Streptomycens renacea; Soil, decaying vegetation | [33]         |
| Chlorotetracycline      | Antibiotic                       | Cl (01)                 | Streptomycens aurificus; Agricultural soil | [34]         |
| Gnisalofavin            | Antifungal drug                  | Cl (01)                 | Penicillium griseofurum; Soil           | [35]         |
| Pyoluteorin             | Antibiotic                       | Cl (02)                 | Pseudomonas aeruginos; Rhizosphere soil | [36]         |
| Fluoroacetic Acid       | Pesticide                        | F (01)                  | Streptomycens catflepe; Soil            | [37]         |
| Pyrrolnitrin            | Antifungal antibiotic            | Cl (02)                 | Burkholderia pyrocens, F. fluorescense, Serata phymathica; Rhizosphere soil | [38]         |
| Nucleosidin             | Nucleoside antibiotic            | F (01)                  | Streptomycens calicus; Soil             | [39]         |
| Vancomycin              | Antibiotic                       | Cl (02)                 | Amycolatopsis orientalis; Soil          | [40]         |
| 2-Chloropentostatin     | Nucleoside antibiotic            | Cl (01)                 | Actinomadura sp.; Soil                  | [41]         |
| Napyradiomycin          | Antibiotic                       | Cl (02)                 | Chainia rubra; Soil                     | [42]         |
| Calicheamycin B         | Cytoxin                          | Br (01)                 | Micromonopora echinopus; Rhizosphere soil | [43]         |
| Pyromycopins B          | Antibiotic                       | Cl (01)                 | Streptomycens regoups;; Soil            | [44]         |
| Pentabromopseudilin     | Marine antibiotic                | Br (05)                 | Pseudomonas bromoautalis; Coastal area  | [45]         |
| Cryptophycin A          | Anticancer                       | Cl (01)                 | Cyamtheteran; Terrestrial, aquatic habitat | [46]         |
| 2-Chloro-4-Nitrophenol  | Fungicide                        | Cl (01)                 | Streptomycens canecolor; Rotting wood or plant debris | [47]         |
| 3,5 Dichloro-Hexanophenone | Inhibits fruiting body formation | Cl (02)                 | Dietistelium discoid; Decoying peack    | [48]         |
| Rebecamycin             | Weak Topoisomerase I Inhibitor, antitumor | Cl (02)                 | Streptomycens sp.; Rhizosphere, agricultural soil | [49]         |
| Chlorotetracycline      | Antibiotic                       | Cl (01)                 | Streptomycens aureofuscens; Sanborn field | [50]         |
In halometabolites, the halogen atom from halides ions (Cl\(^{-}\), Br\(^{-}\), I\(^{-}\) and F\(^{-}\)) is incorporated in organic compound with halogenation catalyzed by halogenase. Metabolites having bromine and iodine are mostly secreted by invertebrates and algae from marine habitats. Organisms from sea water habitat have comparatively more bromine content, while chlorinated metabolites were dominant in terrestrial species. Besides, fluorinated metabolites were also synthesized by few higher plants [30]. The 200-fold increase in the number of secondary metabolites with halo-molecules has been seen due to extensive research for antibiotics from marine habitats. It may have happened because incorporation of a halogen moiety potentiates more bioactivity and facilitates bioavailability of molecules [49]. Furthermore, the prevalence of halogen (Cl- or Br-) can offer a chemically reactive and orthogonal handle for selective modification through cross coupling chemistry [50]. The most common halogen found in secondary halometabolites is chlorine followed by bromine, while iodine and fluorine are considerably low [49,51]. Of these, chlorinated halometabolites has more advantages of being amenable to chemical modification for tailor-made bioactivity and increased drug efficacy [52]. The recent surge of interest in halometabolites seems to be due to their potentialities as effective alternative to current antifungal agents and, therefore, the pyrrolnitrin metabolite of soil microbial habitat holds promise.

3. Pyrrolnitrin (PRN)

Pyrrolnitrin [3-chloro-4-(2-nitro-3-chlorophenyl) pyrrole] is a phenylpyrrole derivative containing two chlorine atoms and a nitro group. PRN, isolated from *Pseudomonas pyrocinia* and various other pseudomonads, was classified as halometabolite in as early as 1964 [38]. Later, the compound was biosynthesized using tryptophan as supplement in the medium [53] and chemically synthesized by Nakano et al [54]. Biosynthesis of PRN in *Pseudomonas aureofaciens* ATCC 15926 has shown that L-tryptophan is a direct precursor (Figure 1) [53]. However, Hammil et al. [55] obtained high yield of PRN in D-tryptophan amended medium. Tryptophan analogs amended in the fermentation medium can also yield a series of PRN-like derivatives [56] (Table 2) with low antimicrobial activity than the native parent compound.
| IUPAC Name | Common Name | Structure | Extinction Coefficient $\lambda_{max}$ MeOH (log ε) | Molecular Formula | Molar Mass/Exact Mass: Molecular Weight |
|------------|-------------|-----------|---------------------------------------------------|------------------|----------------------------------------|
| 3-(2-amino-3-chlorophenyl)-pyrrole | Mono-chloro-amino-pyrrolnitrin (MCA) | ![Structure](image1) | - | C$_{10}$H$_{6}$ClN$_2$ | Exact Mass: 192.05 Mol. Wt.: 192.64 |
| 3-chloro-4(2-amino-3-chlorophenyl)-pyrrole | Di-chloro-amino (DCA) (amino-pyrrolnitrin) | ![Structure](image2) | 212 (4.46) | C$_{10}$H$_{8}$Cl$_2$N$_2$ | Exact Mass: 226.01 Mol. Wt.: 227.09 |
| 2, 3 dichloro-4-(2-amino-3-chlorophenyl)-pyrrole | Tri-chloro-amino (TCA) | ![Structure](image3) | 212 (4.64) | C$_{10}$H$_{7}$Cl$_3$N$_2$ | Exact Mass: 259.97 Mol. Wt.: 261.53 |
| 3-chloro-4(3-chloro-2nitro-phenyl)-1H pyrrole | Pyrrolnitrin (PRN) | ![Structure](image4) | 212 (4.39) | C$_{10}$H$_{6}$Cl$_2$N$_2$O$_2$ | Exact Mass: 255.98 Mol. Wt.: 257.07 |
| 2, 3 dichloro-4-(2-nitro-3-chlorophenyl) pyrrole | 2-chloro-pyrrolnitrin (2-CPRN) | ![Structure](image5) | 212 (4.47) | C$_{10}$H$_{5}$Cl$_3$N$_2$O$_2$ | Exact Mass: 289.94 Mol. Wt.: 291.52 |
| 2-(2-Heptenyl)-3-methyl-4(1H) quinolone | - | ![Structure](image6) | - | C$_{17}$H$_{21}$NO | Exact Mass: 255.16 Mol. Wt.: 255.35 |
| 2,3 dichloro-4-(2-nitrophényl) pyrrole | Iso-pyrrolnitrin | ![Structure](image7) | - | C$_{10}$H$_{8}$Cl$_2$N$_2$O$_2$ | Exact Mass: 255.98 Mol. Wt.: 257.07 |
| 3-chloro-4(2-nitro-3-chloro-6-hydroxyphenyl) pyrrole | Oxy-pyrrolnitrin | ![Structure](image8) | - | C$_{10}$H$_{8}$Cl$_2$N$_2$O$_3$ | Exact Mass: 271.98 Mol. Wt.: 273.07 |
Structurally, PRN possesses benzene and pyrrole rings with chlorine atoms on both of them and nitro and chlorine units to form an unusual natural skeleton. It has chlorine moiety to contribute more towards biological activity \[57\] in comparison to its bromine derivative \[58\]. Consequently, several natural congeners of PRN such as amino-pyrrolnitrin, iso-pyrrolnitrin, 2-chloropyrrolnitrin, oxy-pyrrolnitrin, 4-fluoropyrrolnitrin, and 3-fluoro-3-dechloropyrrolnitrin have been reported. Brominated derivatives of PRN can be synthesized by replacing chlorine ion with bromine in the presence of sodium bromide.

### Figure 1
Biochemical steps in the synthesis of pyrrolnitrin. 7-chlorotryptophan is formed from tryptophan due to flavin-dependent halogenation catalyzed by the enzyme tryptophan 7-halogenase (PrnA). Further, the enzyme PrnB (monodechloroaminopyrrolnitrin synthase catalyzes formation of monodechloroaminopyrrolnitrin from 7-chlorotryptophan while the enzyme PrnC leads to catalytic reaction for the conversion of monodechloroaminopyrrolnitrin into aminopyrrolnitrin. In the last step, aminopyrrolnitrin is converted to pyrrolnitrin with the help of the enzyme PrnD (aminopyrrolnitrin oxygenase).

#### 3.1. Pyrrolnitrin: Chemical Synthesis

PRN is positive towards Ehrlich's reagent where pyrrole ring gets condensed with p-dimethylaminobenzaldehyde to form the violet color complex. Pauly’s coupling reaction yields red color \[59\] and gives a negative reaction to the ferric chloride nitro group detection test. PRN can be oxidized by chromic acid to form corresponding compound which on oxidation with permanganate, yields carboxylic acid \[38\].

Modern synthetic targets for chemical synthesis require regiospecific polysubstituted aromatic or heteroaromatic components \[60\]. PRN is chemically synthesized by α-block of pyrrole ring and 2-nitro-3-chloroacetophenone, and subsequent chlorination at 4-position and oxidation of methyle using sulfurichloride followed by decarboxylation \[54\]. In another approach, 2-Methyl-4-(2-nitro-3-chloro-phenyl)-5-ethoxycarbonyl-pyrrole was prepared in various steps from 2-amino-3-chloro-toluene \[61\]. One of the most versatile synthetic approach for PRN allowed access to analog compounds such as monodechloroaminopyrrolnitrin and aminopyrrolnitrin. This step facilitated PRN synthesis using Suzuki–Miyaura cross-coupling of an appropriately halogenated pyrrole pinacolboronate ester with halogenated arlypyrroles using 2,6-disubstituted nitrobenzenes or 2,6-disubstituted anilines \[62\]. Palladium-catalyzed coupling of 1-(triisopropylsilyl)-3-substituted pyrroles with arylhalides has also been described \[63\]. However, chemical synthesis of PRN makes synthetic route cost extensive and pose threat to the environment \[62\]. Furthermore, the chemical process utilizes noxious chemicals, high temperature and pressure, more energy and yield poor regioselectivity with lack of public acceptability \[49\]. Thus, chemical industries prefer microbial species for more selective, greener and cost-effective approach for synthesizing PRN.
3.2. Microbial Pyrrolnitrin Production and Recovery

Microbial synthesis of PRN is easy, reliable and eco-friendly and requires low-cost medium constituents, ambient conditions for growth and production, the least additional energy requirements and minimum expensive equipment. This is the major reason microbial synthesis of PRN has become the preferred alternative to chemical processes [49]. After initial isolation of PRN from *Pseudomonas pyrocinia* [38] and thereafter reports from different fluorescent and non-fluorescent *Pseudomonas* species [53,64], several strains of *Burkholderia cepacia*, *Corallococcus exigus*, *Cystobacter ferrugineus*, *Enterobacter agglomerans*, *Myxococcus fulvus*, *Serratia* spp. and *Actinosporangium vitaminophilum* have been classified to produce PRN in varying quantities [65–68]. *Serratia plymuthica* [69] and *S. ruhidaea* [70] are identified for enhanced production of PRN. Recently, a strain belonging to *Burkholderia cepacia* complex, JKB9, showing broad-spectrum antifungal activity, was held responsible for suppressing growth of *Phytophthora capsici*, *Fusarium oxysporum* and *Rhizoctonia solani* [71]. This strain, which has shown stronger antifungal activity than *Burkholderia* strains KCTC2973 and ATCC25416 against *Phytophthora* blight, was confirmed for PRN production using thin layer chromatography (TPC), high performance liquid chromatography (HPLC) and Nuclear Magnetic Resonance (NMR) spectrometric studies. Complete genome sequencing of *Burkholderia pyrocinia* 2327T revealed insights into the cells possessing antibiotic capabilities for the biosynthesis of PRN [72]. Cloning of gene clusters responsible for encoding enzymes involved in the production of pyrrolnitrin in organisms has greatly helped in marking of the biosynthetic routes. Using an antibiotic producing strain of *P. fluorescens* [73] cloned four gene clusters to elucidate biochemistry of these molecules and to link it with the enzymes that may offer the routes for the synthesis of new chemical structures. Earlier, *prnABCD* operon from *P. protegens* Pf-5 was co-expressed in tomato plants with universal vector IL-60 and successfully demonstrated resistance to damping-off disease caused by *R. solani* [74].

Microbial wild type strains secrete PRN in low quantity (Table 3) and production varies with the medium constituents. *P. aureofaciens* ATCC 15926 strain when grown in minimal medium, secreted PRN in low concentration (≤0.3 µg mL⁻¹). Even optimized variation of constituents in growth medium could not increase PRN production. However, the production enhanced by 30-fold when *P. aureofaciens* ATCC 15926 was mutated with N-methyl-N’-nitro-N-nitrosoguanidine [75]. Addition of DL-tryptophan (1 mg mL⁻¹) in CMM medium also doubled PRN production after 120 h but additional amount of tryptophan resulted in less yield [76].
Table 3. Characteristics of PRN production by different microbial species inhabiting several ecohabitats.

| Sr. no. | Producer | Habitat | Medium | Physical Condition | Incubation Period (Days) | Concentration | Significance | Reference |
|---------|----------|---------|--------|-------------------|--------------------------|--------------|-------------|-----------|
| 1.      | *Pseudomonas pyrrocinia* | - | Bouillon Medium | - | - | ND | Antibiotic, antifungal nature | [26,38] |
| 2.      | *P. aureofaciens, P. fluorescens, P. multivorans* | - | CMM, Synthetic C, E | 27 °C, shaker | 7 | 0.32-126 (µg mL⁻¹) | PRN widespread in groups of *Pseudomonas* | [64] |
| 3.      | *P. aureofaciens* | - | CMM | 27 °C, shaker | 5 | 9.5 to 50 (µg mL⁻¹) | Production of substituted PRN from Tryptophan analogs | [56] |
| 4.      | *P. aureofaciens* | - | CMM | 30 °C, shaker | 5 | 18.35-19.9 (µm) | Possible pathway discussed | [77] |
| 5.      | *Pseudomonas cepacia* B37w (NRRL B-14658) Rhizosphere | Sabouraud Maltose Broth | - | - | 6 | 2.133 (mg L⁻¹) | Efficacy against *F. Sambucinum* incited potato dry rot disease | [59] |
| 6.      | *Pseudomonas cepacia* LT4-12- W | Apple leaves | Mineral Salt, Nutrient Broth, Kings medium B | 27 °C, 200 rpm | 7 | 1) MS: 51.50 (mg L⁻¹) 2) NB: 7.20 (mg L⁻¹) 3) KMB: 5.50 (mg L⁻¹) | Production of phenylpyrrole metabolites with respect to time | [78] |
| 7.      | *B. cepacian* | - | Mineral Salt | 27 °C, shaker | 7 | ND | Delays postharvest fruit rot in strawberries | [79] |
| 8.      | *Enterobacter agglomerans* IC1270 | Grapes rhizosphere | Potato Dextrose Agar | Incubated on agar plate | 5 | ND | Possible role of a combination of Chitinases and pyrrolnitrin in antagonism | [65] |
| 9.      | *B. cepacia* NB-1 | Ponds in botanical garden of Tübingen, Germany | Minimal medium | 27 °C, aeration rate 0.5 vvm, stirrer speed 150 rev min⁻¹, pH 7.0 | 5 | 0.54 (mg L⁻¹) | PRN block ETS Neurospora crassa 74 A; inhibition of Streptomycine spp. | [66] |
| 10.     | *Burkholderia cepacia* c5.5B (ATCC 55344) Wild Type | Soil sample, North Carolina | Nutrient broth, Mineral salt | 25 °C, at 200 rpm, pH 5.8 | 5 | NB: 35.59; MS: 28.54 (mg 10¹² cfu) | Biocontrol of Rhizoctonia stem rot of poinsettia | [80] |
| 11.     | *Pseudomonas fluorescens* psd | Roots of Vigna mungo | Standard succinate medium (SSM) | - | - | ND | Biocontrol property of plants protected from strain | [81] |
| 12.     | *Pseudomonas chlororaphis* O6 | Nutrient broth, Mung bean medium | 28 °C 200 rpm | - | 1.7 (µg mL⁻¹) | Regulation by glucose of PRN production influenced biocatalysis of tomato leaf blight disease | [82] |
| 13.     | *Acinetobacter haemolyticus* A19 | Wheat rhizosphere | Luria broth | - | 2 | 15 (mg L⁻¹) | Plasmid-mediated pyrrolnitrin production by *A. Haemolyticus* A19 | [83] |
| 14.     | *Pseudomonas chlororaphis* strain PA23 | M₉ medium + 1 mm MgSO₄ + 0.2% glucose | - | - | 5 | ND | Nematicidal and repellent activity against Caenorhabditis elegans | [84] |
| 15.     | *Serratia marcescens* ET17 | Tea rhizosphere | Semi-solid pigment producing media | 30 °C | 8 | ND | Effective reduction of root-rot disease tea plant on talc-based formulations; Plant growth promoting activity | [85] |

CMM: Citrate minimal medium; ND: not determined; NB: Nutrient broth; MS: Murashige-Skoog medium; cfu: colony forming units; KMB: King’s medium-B.
Besides intracellular production of PRN from *Pseudomonas* spp., the excretion of the compound was also detected in the supernatant of fermented medium of *Serratia marcescens* strain ETR17 [85]. B. cepacia yielded 0.54 mg L\(^{-1}\) of PRN in monosodium glutamate medium at 27 °C as quantified by preparative HPLC [66]. Initially, Elander et al. [64] reported that only 27.58% *Pseudomonas* spp. secreted PRN in shake flask fermentation propagated in CMM, C, or E media. The authors concluded that *P. multivorans* C653 (ATCC 17760) showed maximum PRN production in medium C, followed by E and then CMM. *P. aureofaciens* was shown to secrete moderate PRN in CMM medium (40–80 µg mL\(^{-1}\)). The PRN concentration increased in D-tryptophan amended medium where it was incorporated in the biosynthesis of PRN.

While growing *P. aureofaciens* in isotopically labeled tryptophan (at different positions) containing medium, Martin et al. [86] demonstrated that amino nitrogen of D-tryptophan became the nitro group of PRN. The two chlorine atoms in PRN, C3 of side chain became pyrrole and C2 of the indole nucleus got retained during biosynthesis (Figure 1). Furthermore, Chang et al. [77] confirmed that H-2 and H-α of the indole and side chain give rise to H-5 and H-2 of PRN, respectively, and, thus, proposed that L-tryptophan is the immediate precursor in PRN biosynthetic pathway. PRN formation using labeled tryptophan showed that L- rather than D-tryptophan was the immediate precursor of PRN [87]. 7-chloroindole-3-acetic acid, 3-chloroanthranilate detected in fermented medium revealed that 7-chlorotryptophan served as a common precursor for PRN [88].

Variety of production media and their pH remained a key parameter to influence PRN secretion. Shake flask fermentation of *P. cepacia* LT4-12-W revealed that the final yield (at 168 h) of PRN almost doubled at pH 5.8. Amendment of MS medium with glutamate salt of sodium yielded 60.50 mg mL\(^{-1}\) of PRM secretion [89]. The effect of different physicochemical conditions on plasmid-mediated PRN secretion has also been reported from *Acinetobacter haemolyticus* A19 isolate from wheat rhizosphere [83].

Recovery strategy of PRN involves cell growth in appropriate medium, extraction in acetone followed by removal of oily matter from concentrated acetone solution using petroleum benzene [38]. From fermented broth at pH 10 or 11 (6 mL) with NaOH, cell pellet centrifugation following sonication with acetone (600 µL) for 1 min, separation of acetone supernatant, re-extraction of pellets again in acetone (300 µL) and drying of acetone extract also yield PRN extract [59]. Further, fermented cultures were extracted after 48 h with equal volume of ethyl acetate [90] and centrifuged. Pellets sonicated twice with ethyl acetate (5 mL) for 3 min then recovery of organic phase [91] resulted in PRN rich dried extract [92]. Majumdar et al. [83] reported lysis of 18 h culture of *Acinetobacter haemolyticus* A19 using 1% SDS followed by sonication for 5–15 min and supernatant collection for PRN. In the case of bioactivity and characterization study, chromatographic separation techniques such as column chromatography and flash column with different mobile phases were explored (Table 4).
Table 4. Purification of pyrrolnitrin using various separation techniques with different solvent systems.

| Matrix                  | Column                                | Organic Phase                                | Detection               | Reference |
|-------------------------|---------------------------------------|----------------------------------------------|-------------------------|-----------|
| Silica gel G            | 35 cm × 1.5 cm                        | Chloroform: methanol (9:1)                   | -                       | [83]      |
| Silica gel (40 µm)      | 35.6 cm × 1.75 cm                     | Benzene: hexane (2:1); Benzene; Benzene: acetone (1:1); Acetone; methanol | TLC - bioautography     | [59]      |
| Silica gel (60 µm)      | -                                     | Chloroform: hexane (1:1, 1.5:1, 2:1, 5:1 (v/v); chloroform; chloroform-acetone (5:1, 1:1) (v/v); acetone | Bioassay with *R. solani* | [65]      |
| Sephadex LH-20          | -                                     | Methanol                                     | pHPLC                   | [66]      |
| Silica gel 60 (0.015–0.040 mm; Merck) | -                                   | Dichloromethane then methanol                | TLC                     | [93]      |
| Silica gel (H60)        | -                                     | Dichloromethane                              | Bioautography           | [94]      |
| Silica gel              | (20 × 170 mm, Wakogel C-200)          | Benzene, 10% ethyl acetatobenzene, 20% ethyl acetate benzene and finally ethyl acetate | TLC                     | [95]      |
| Silicic acid            | (240 × 22 mm)                         | Diethyl ether and methanol                   | -                       | [96]      |
| -                       | RP C-18 flash                         | Water and methanol                           | TLC                     | [97]      |
| -                       | RP C-18 (MPLC)                        | 50% to 100% aq methanol                      | HPLC                    | [97]      |
| Silica gel (60 µm)      | -                                     | Toluene                                      | -                       | [98]      |

TLC: thin layer chromatography; HPLC: high performance liquid chromatography; Aq.: Aqueous; MPLC: medium pressure liquid chromatography; RP: Reverse phase; pHPLC: preparative HPLC.
3.3. Analytical Characteristics of Pyrrolnitrin

PRN is chemically substituted with 3-phenyl pyrrole derivative containing two chlorine atoms and a nitro group [57]. The compound is a pale-yellow crystal [38], 3-chloro-4-(3-chloro-2-nitrophenyl)-1H-pyrrole, a phenylpyrrole molecule having a chemical formula of $\text{C}_{10}\text{H}_{6}\text{O}_{2}\text{N}_{2}\text{Cl}_{2}$ and molecular weight 257.07 gmol$^{-1}$. The melting point of PRN, which is sparingly soluble in water, petroleum ether and cyclohexane, but more soluble in ethanol, butanol, ethyl acetate, ethyl ether, and chloroform, is 124.5°C. Elemental analysis of the compound reflected C, 46.71%; H, 2.33%; O, 12.45%; N, 10.89%; and Cl, 27.68% [38]. PRN separation was achieved by different methods like chromatography TLC and HPLC [65,82] while structural features have been elucidated using Fourier transform infrared spectroscopy (FTIR) [93], nuclear magnetic spectroscopy (NMR $^1\text{H}$ and $^{13}\text{C}$) [89] and mass spectroscopy (LC-MS and GC-MS) [99].

Separation of PRN from bacterial media extract using TLC utilized various stationary phases such as silica gel G, GF$_{254}$, 60 F$_{254}$, KCl8 F, C18 Glass and several mobile phases. PRN can be detected on TLC under UV transilluminator [83,100] and visualized by spraying diazotized sulfanilic acid (DSA) or Pauly’s, Ehrlich’s and van Ûrk’s reagent to develop maroon and violet color, respectively [101,102] or H$_2$SO$_4$ on Silica Gel G plate [64]. The $R_f$ value for various TLC system served to identify PRN from different bacterial species. The compound has been analyzed by retention time in gradient HPLC system [65] but isocratic solvent system of 45% water, 30% acetonitrile, and 25% methanol also separated pyrrolnitrin at 252 nm in preparative HPLC [102]. Modifications in the polarity of solvents, mobile-stationary phase and elution methods are key strategies to quantify PRN using HPLC (Table 5). Yellow colored PRN molecule isolated from Pseudomonas pyrrocinia absorbs at 252 nm with molar extinction coefficient of $\varepsilon = 7500$ in ethanol [26]. Myxobacterial PRN also showed $\lambda_{\text{max}}$ at 252 nm in methanol [94]. Functional group stretching in FTIR vary with different PRN derivatives due to its structural features. Typical bond stretching at 1530 and 1375 cm$^{-1}$ characterized for nitro group [38] while 3489 cm$^{-1}$ represent pyrrole ring. Similarly, PRN isolated from supernatant of fermented medium inoculated by Myxococcus fulvus strain Mx f147 indicated infrared spectrum to confirm pyrrole ring (3460), nitro group (1530 and 1375), CH$_3$ (stretch) (1460) and C=C aromatic weak intensity (1600) [94]. Mass spectroscopy (MS) of PRN is ascertain using different ionization techniques. MS of PRN isolated from Pseudomonas cepacia B37w showed molecular ion at m/z 256 with the formula C$_{10}$H$_6$C$_{12}$N$_2$O$_2$ [59]. Electrospray mass spectroscopy (negative ion spectrum) of PRN further confirmed (mass-to-charge ratio; m/z) at 256 [66]. High-resolution mass spectrometry of the two molecular ions gave m/z 255.9826 and 257.9777, respectively, indicating the molecular formula C$_{10}$H$_6$N$_2$O$_2$$^{35}$C$_{12}$ and C$_{10}$H$_6$N$_2$O$_2$$^{35}$Cl$^{37}$Cl [99].
Table 5. Several HPLC methods adopted to separate and quantify pyrrolnitrin from microbes using different solvent system.

| Column | Flow Rate (mL/min) | Solvent System | Detector | Retention Time (min) | References |
|--------|-------------------|----------------|----------|----------------------|------------|
| RP 18  | 2                 | Methanol: water (70:30; v/v) | -        | -                    | [103]      |
| 50 mm × 4.6 mm I.D. guard | 1.0 | Acetonitrile: methanol: water (1:1:1) | UV (254 nm) | 10 | [78] |
| Rainin Dynamax C18 (21.4 × 250 mm) | - | Acetonitrile: water (3:2; v/v) fractions collected at 9.5 to 12.5 min and re-chromatographed on a silica column eluted with chloroform: hexane (1:1; v/v) | - | 13.5 | [79] |
| C-18 column, 5 µm | - | Isocratic acetonitrile: methanol: water (1:1:1) | - | - | [59] |
| Hypersil octyldecyl saline (2.1 mm diameter by 10 cm) | - | Water: methanol from 0%: 100% and gradually changing up to 100%: 0% | - | between 10-15 | [104] |
| Reverse phase 18 | 0.7 | 0 min 50% methanol in water 15 min 100% methanol in water 17 min 100% methanol in water 20 min 50% methanol in water 25 min 50% methanol in water | UV (252 nm) | 15.4 | [65] |
| C-18 reverse phase (125 × 4.6 mm) | - | Methanol: water (70:30; v/v) | UV (252 nm) | - | [105] |
| - | 1.0 | 2-min initialization at 10% ACN: 0.1% TFA; 20-min linear gradient to 100% ACN: 0.1% TFA | 990-photodiode array detector | - | [91,106] |
| Nucleosil C-18 | - | Acetonitrile: water (20 to 100%) | - | 27.5 | [66] |
| RP C-18 column | 1.0 | Isocratically 45% water: 30% acetonitrile: 25% methanol | - | - | [102] |
| C-18 RP column | - | 10% acetonitrile: water (v/v) (both acidified with 0.1% amino acid) run for 2min linear gradient 100% acetonitrile (acidified with 0.1% amino acid) | - | 18 | [107] |
| Gemini C18 column (100 × 4.6 mm; 5 mm particle diameter) | 1.0 | Isocratically 45% acetonitrile: 35% water: 20% methanol | Dionex AD20 (Dionex, Sunnyvale, CA) (225 nm) | - | [84,108] |
| Cosmosil C18 | 0.7 | 18 min linear gradient from 50 to 100% methanol and 0.1% trifluoroacetic acid in methanol | - | - | [82] |
NMR spectroscopy is widely used for analytical measurement of microbial metabolites. The PRN is confirmed by NMR spectrum [59] with values: (i) 1H NMR: H-2 and H-5: 6.82 (m, 2H); H-5′: 7.41-7.53 (m, 3H); NH: 8.29 (br s, 1H); and (ii) 13C NMR δ value: 111.9 indicated C-3, 115.4 for C-4, 116.6 for C-5, 117.4 meant for C-2, while 124.8, 127.6, 128.6, 130.1, and 130.3 designated for C-3′, C-1′, C-6′, C-4′ and C-5′, respectively. Chemical shift (δ) values at 6.81 (m, 2H) indicate the presence of H-2 and H-5, 7.41: H-6′, 7.43: H-5′, 7.52: H-4′, 8.38: NH [89]. NMR spectrum of purified PRN secreted by plasmid-mediated A. haemolyticus A19 revealed the values δ: 6.2–6.6 (m, 2H, H-2, H-5), 6.77 (q 1H, H6), 7.03 (m, 1H, H-4), 7.38 (m2H, Ha, Hc) compared with standard 1H NMR spectrum of [65]. PRN synthesized from Myxococcus fulvus strain Mx f147 showed 13C NMR spectrum (in acetone-d6; Bruker 400 MHz) [94]. Structural investigation of PRN with X-ray analysis revealed the presence of two molecules with observed density of 1.74 g/cm³ that lie opposite to each other about the center of symmetry. It further confirmed the location of two Cl atoms in the asymmetric unit with 3D Patterson function, dihedral angle of the pyrrole, the benzene rings and chlorine substitution on pyrrole ring located apart from the nitro group [109].

3.4. Biochemistry of Pyrrolnitrin

Microbial synthesis of PRN requires D-tryptophan, but cost of precursor amino acid and intracellular secretion limits its large-scale production. The NO₂ group is derived from anthranilic acid, phenylalanine and tryptophan that could serve as a precursor for PRN secretion [57]. However, anthranilic acid and L-phenylalanine usually decrease PRN secretion in P. aureofaciens and B. cepacia [66], while tryptophan stimulates PRN production [57,101]. In the medium, L-tryptophan gets quick intake within the cells than the D-isomer but addition of D-isomer could not yield more PRN secretion [110]. In actinomycetes, D-tryptophan enhances secretion of PRN when added separately in the culture medium [101] and maximum accumulation was observed at stationary phase after 120 h [66,101]. It indicated that the L-isomer of tryptophan enter cells quickly and participate in the protein synthesis, while D-tryptophan enter slowly and available at the time of antibiotic secretion [55]. Besides, L-glutamic acid amended medium showed maximum antifungal activity, which substantially declined with the addition of L-tryptophan, L-valine, L-serine, L-phenylalanine and L-cysteine [66]. In brief, D-tryptophan and L-glutamic acid are more direct precursors of PRN than any other amino acids.

PRN biosynthesis was unraveled in P. aureofaciens [77,101]. Later, genes (prnABCD operon) and corresponding enzymes involved were delineated in P. fluorescens BL915 (Figure 2) [90,111]. The biosynthesis of PRN occurs in four sequential steps: chlorination by prnA, rearrangement and decarboxylation by prnB, chlorination by prnC and oxidation by prnD enzyme (Figure 1). This involves regioselective halogenation of tryptophan through the addition of chlorine into D-tryptophan by tryptophan 7-halogenase (prnA) following nucleophilic and electrophilic reactions [112] and activation of intermediate lysine-chloramine species as the first step [113,114]. Further, the reaction catalyzed by prnB shows structural similarity with two-domain indoleamine 2,3-dioxygenase enzyme (IDO) and involves several intermediary steps. The second step forms a binary complex that combines with L-tryptophan or 7-Cl-L-tryptophan to create a ternary complex. The third step in PRN biosynthetic pathway of P. fluorescens leads to catalytic conversion of mono-chloro-deamino-pyrrolnitrin into amino-pyrrolnitrin by regioselectivity using halogenating and chlorinating enzyme [115]. In the last step, prnD catalyzes the oxidation of amino group of aminopyrrolnitrin to nitro group and thus forms PRN [90,111,116].
Aminopyrrolnitrin oxidase or arylamine oxygenase (rieske N-oxygenase) catalyzes oxidation of an arylamine into the arylnitro group. Except prnB, tryptophan-7-halogenase (prnA), monodechloroaminopyrrolnitrin (prnC) and aminopyrrolnitrin oxidase (prnD) enzymes require flavin reductase (prnF) gene located close to the prnABCD operon which is considered as a part of the cluster [117]. Bioinformatics clubbed with the biochemical tools identified the role of prnF gene in prnD-catalyzed unusual arylamine oxidation in Pseudomonas fluorescens Pf-5 [118]. The prnF and prnD genes form a two-component oxygenase system, in which the gene product enzyme prnF supplies the reduced flavin to prnD. The prnF requires NADH as an electron donor to reduce FAD so that reduced FAD supplies electrons from NADPH to the prnD oxygenase component through protein-protein interactions in order to protect the flavin from oxidation.

The prnF gene having molecular mass of 17kD with GC content of 62%, encodes for a polypeptide chain of 160 amino acids. The enzyme belongs to flavin:NAD(P)H reductases family with part of two-component monooxygenase systems and its C-terminal region possesses highly conserved GDH motif for NAD(P)H binding [119]. It resembles with PheA2, SnaC, VlmR, ActVB and HpaC with 31.5%, 28.6%, 26.4%, 25.6%, and 25.5% amino acid identity, respectively [120–126].

3.5. Pyrrolnitrin Derivatives

Several halogen variations of the PRN molecule have been isolated in the past in the form of bromo-analogs of pyrrolnitrin from fermentation of Pseudomonas aureofaciens in sodium bromide with low antifungal activity [58]. In addition, 2-chloropyrrolnitrin contain an additional chlorine atom which possesses about 10% of the antifungal activity of PRN [55]. The pyrrolomycin (B, C, D, E, F₁, F₂a, F₂b, and F₃) derivatives encompass a chlorine or bromine atom at the 3-position of the pyrrole ring, and either two chlorine atoms at positions 4 and 5 or one chlorine and one bromine at any of these positions have shown significant antifungal activity [57]. Novel oxidized derivatives of pyrrolnitrin including two new pyrrolnitrin analogs, namely 3-chloro-4-(3-chloro-2-nitrophenyl)-5-methoxy-3-pyrrolin-2-one and 4-chloro-3-(3-chloro-2-nitrophenyl)-5-methoxy-3-pyrrolin-2-one, were reported from B. cepacia K87 [97].
Furthermore, number of de-chloro and de-nitro derivatives of PRN and the isomers were synthesized by cyclization of enamine reaction, hydrolysis, carboxylation and Mannich’s reaction [127]. The strongest antifungal activity of PRN and its analogs resulted when it got unsubstituted by ester group at any position. The antifungal activity become more stronger when shift of NO$_2$ group was increased. Few PRN derivatives such as denitropyrrlnitrin (3-chloro-4-(3-chlorophenyl), bromo analog: 3-chloro-4-(3-bromophenyl)pyrrole) and trifluoromethyl derivative (3-chloro-4-(3-trifluoromethyl) pyrrole) were strong antimicrobials. However, among all the analogs homologous to NO$_2$ group of pyrrolnitrin, PRN has remained the strongest biologically active compound. The UV irradiation of 2-(pyrrol-3-yl)nitrobenzene moiety of PRN in an anhydrous aprotic solvent yielded 7,4'-'dichlorospiro(1,3-dihydrobenzo(c)isoxazole-3,3'-'pyrrolin-2'-'one) by the intramolecular oxidation. Hence, the photodegradation of PRN depends on aqueous reaction media and the nature of its excited state [98].

4. Applications of Pyrrolnitrin

4.1. Biological Activity

Structure–activity mechanism reveals that the primary target of PRN lies in the cell membrane to impede protein, RNA, DNA synthesis and uncouple the normal electron flow in the respiratory electron transport chain [128]. The metabolite has demonstrated biological activity at low concentration and act as an uncoupler of oxidative phosphorylation in *Neurospora crassa*. High concentration of PRN causes impairment of electron transport in flavin region and cytochrome c oxidase; accumulation of glycerol; synthesis of triacyl glycerol leading to leakage of cell membrane and inhibition of cell growth; in vitro activity against bacteria and fungi in the range of 1–100 µg mL$^{-1}$; in vitro activity against leukemia and melanoma cell lines; and moderate antymycobacterial activity at 8 µg mL$^{-1}$ [129]. The halometabolite was used as a drug lead for fenipoclonil and fludioxonil synthesis [130]. The amino derivative of PRN was identified as an androgen receptor antagonist [131]. PRN has the unique property to persist actively in the soil over a month, and can be readily diffused and slowly released after lysis of host bacterial cell [132]. However, the compound is sensitive to decomposition due to light [98].

Inhibitory effect of PRN is seen on the mitochondrial electron transport system of *Neurospora crassa* 74A [66]. Studies using N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride (TMPD) confirmed that PRN block transfer of electron between the dehydrogenases and cytochrome c-oxidase components of the respiratory chain. At low concentrations, PRN uncouples oxidative phosphorylation in *Neurospora* mitochondria and impedes electron transport in both the Flavin region and cytochrome C oxidase at high concentration [133]. PRN also function as a signal molecule, beyond its role as a bioactive molecule to suppress fungal and affected cell motility [134]. Antifungal activity of the compound increased at pH 6.0, became maximum at pH 10 or 11 and declined after pH 11. Temperature influence on antifungal activity was maximum at 28°C. Similarly, 2% NaCl content in the medium showed maximum activity. Such studies indicated more scope for medium modifications for obtaining maximum PRN production followed by maximizing biological activity of the compound.

The quorum-sensing system related regulation of PRN is reported in a chitinolytic bacterium *Serratia plymuthica* strain HRO-C48, that protects oilseed rape crop from *Verticillium wilt* [102]. The mutant deficient in PRN production shown the ability to produce the compound in the medium supplemented with chemically synthesized N-hexanoyl-HSL and N-3-oxo-hexanoyl-HSL (OHHL) (100 µM), thus suggesting that quorum sensing (QS) ability regulated PRN biosynthesis. While investigating the role of N-acylhomoserine lactone (AHL)-dependent quorum sensing for expressing antifungal traits, Schmidt et al. [135] found that PRN expression was positively regulated by *CepR* gene at transcription level. PRN is reported to have significant antimicrobial potential (Table 6) against *Streptomyces antibioticus*, *S. violaceoruber*, *Paecilomyces variotii* and *Penicillium puberulum*. However, *S. prasinus*, *S. ramulosus*, *Aspergillus prolificans* and *A. terreus* showed tolerance to pyrrolnitrin. PRN displayed activity against *Ustilago maydis*, *Candida albicans*, *Hansenula anomala*, *Arthrobacter*
oxidans, Bacillus coagulans, B. licheniformis, B. subtilis and B. thuringiensis at low concentrations [66]. Pyrrolnitrin produced by Pseudomonas chlororaphis strain PA23 exhibited nematocidal and repellent activity against Caenorhabditis elegans [84]. Co-culturing P. chlororaphis and C. elegans enhanced expression of biocontrol-related phzA, hcnA, phzR, phzI, rpoS and gacS genes and, thus, contributed to the fast killing of nematode in bacterial interaction.

Table 6. Bioactivity spectrum of pyrrolnitrin against bacteria, fungi and nematodes.

| Sr. No. | Name of Test Microorganism       | PRN (µg mL⁻¹) | Reference |
|---------|----------------------------------|---------------|-----------|
| 1.      | Staphylococcus aureus 209-P       | 6.2           | [38]      |
| 2.      | Escherichia coli                  | 250           | [38]      |
| 3.      | M. tuberculosis CIP 103471        | 4.0           | [129]     |
| 4.      | M. avium CIP 103317              | 8.0           | [129]     |
| 5.      | M. smegmatis CIP 103599          | 16.0          | [129]     |
| 6.      | M. gordonae CIP 6427             | >16.0         | [129]     |
| 7.      | M. marinum CIP 6423              | >16.0         | [129]     |
| 8.      | Candida albicans                  | 1.0           | [38]      |
| 9.      | Saccharomyces cerevisiae          | 10.0          | [38]      |
| 10.     | Cryptococcus neoformans           | <0.78         | [136]     |
| 11.     | Candida albicans                  | 12.5          | [137]     |
| 12.     | Cryptococcus neoformans           | 0.78          | [137]     |
| 13.     | Candida utilis                    | 10.0          | [138]     |
| 14.     | Trichophyton asteroides          | 0.05          | [38]      |
| 15.     | Sporotrichum schenckii           | <0.78         | [136]     |
| 16.     | Penicillium atrovenetum           | 10.0          | [139]     |
| 17.     | P. oxalicum                      | 10.0          | [139]     |
| 18.     | Sporotrichum schenckii           | 3.12          | [137]     |
| 19.     | Blastomyces dermatitidis         | <0.78         | [137]     |
| 20.     | Histoplasma capsulatu            | 0.156         | [137]     |
| 21.     | Sclerotinia sclerotiorum          | 0.01          | [59]      |
| 22.     | Rhizoctonia solani                | 50 (µg/disc)  | [97]      |

Nematode

| Sr. No. | Name of Test Microorganism       | PRN (µg mL⁻¹) | Reference |
|---------|----------------------------------|---------------|-----------|
| 23.     | Caenorhabditis elegans           | 0.1           | [84]      |

Bacterial growth inhibition by PRN forms complex with phospholipids of cell membranes that eventually cease cellular respiration [138]. Furthermore, PRN causes leakage of A260 µm absorbing material inside the cells and impairs synthesis of protein, DNA and RNA [138]. However, in vitro protein synthesis in PRN treated Rhizoctonia solani and Escherichia coli remained unaffected [139]. It bursts protoplast of Bacillus megaterium KM at growth inhibitory concentration [138]. The multitudes and range of activity of PRN makes it a preferred bioactive compound for agricultural chemical sector.
4.2. Agricultural Applications

Phenylpyrroles were proven and effective agents against Trichophyton, Microsporum, Epidermophyton, Penicillium, Candida spp., and several Gram-positive bacteria [57]. Besides, PRN showed activity against soilborne fungal phytopathogens Rhizoctonia solani [140] and Fusarium sambucinum [33] and foliar fungal pathogens Fusarium graminearum, F. culmorum [141], Pyrenophora tritici-repentis [142], Thielaviopsis brasiola, Verticillium dahlia and Alternaria spp [143]. The compound inhibited Gaeumannomyces graminis of wheat, Alternaria brassicaceae and Botrytis cinereal, and partially inhibited Fusarium roseum. Remarkable inhibition of mycelial growth and conidial germination was observed at a PRN concentration of 0.4 µg mL\(^{-1}\) compared to phenazine-1-carboxylic acid at 50 µg mL\(^{-1}\) [144]. Results suggest strong possibility of the compound being a prospective biocontrol agent in the agriculture.

The fungistatic effect of PRN was most distinct against Alternaria sp., Botrytis cinerea, Pythium aphanidermatum, P. ultimum, Rhizoctonia solani, Rhizopus sp. Aspergillus niger, Fusarium oxysporum, Penicillium expansum, and Sclerotium rolfsii [65]. Antibacterial activity was also recorded against Agrobacterium tumefaciens, Corynebacterium insidiosum, Pseudomonas syringae pathovar syringae, and Xanthomonas campestris (Minimum Inhibitory Concentration (MIC) ≥1 µg mL\(^{-1}\)). Organisms such as Clavibacterium michiganense and Serratia marcescens were suppressed at MIC ≥ 10 µg mL\(^{-1}\) [65]. Moderate activity against Gram-positive and Gram-negative bacteria was seen at 12.5–100 mg mL\(^{-1}\) (MIC). Strong toxicity was noticed against fungi, especially trichophytes, Trichophyton asteroids, at MIC of 0.05 mg mL\(^{-1}\). In addition, PRN as a nitro-heterocyclic chemotherapeutic agent exhibited antimycobacterial activity against M. tuberculosis and M. avium [129]. At present, only two synthesized derivatives of PRN, namely fludioxonil and fenpiclonil analogs, were registered as agricultural fungicides in France and Switzerland, respectively. Commercial products of fenpiclonil and fludioxonil include BERET, GALBAS and SAPHIRE, CELEST and MAXIM sold by Syngenta, respectively [145].

PRN found most prolific applications in controlling damping-off disease of cotton and cucumber, tan spot of wheat, storage molds of pome fruits, seedling disease of cotton, dry rot of potato and sclerotinia wilt of sunflower [73]. More usage of the compound lies in its significant antibiotic activity and low toxicity to mammalian species [146]. Wounds on apple and pear were challenged with a conidial suspension of antagonist grey mold B. cinerea and blue mold Penicillium expansum to investigate the efficacy of pyrrolnitrin (6–200 µg mL\(^{-1}\)) to control diseases at 2 and 24 °C after harvest. High concentrations of PRN proved effective at 24 °C on both diseases of apple and pear, while low concentrations appeared effective at cold temperature [147]. Hence, PRN is an attractive strategy to control postharvest diseases on fruits, vegetables and other agricultural products being produced at low temperature conditions. In a preliminary field experiment on strawberries, postharvest treatment with PRN (250 mg L\(^{-1}\)) at low storage temperature delayed development of post-harvest rot by 2–4 days, but did not reduce rate of development [79] and spoilage to acceptable levels.

In greenhouse studies, PRN showed prominent activity against Pyricularia oryzae and Botrytis cinerea [148]. The PRN producer P. chlororaphis O6 has shown antifungal activities both in vitro and in planta [82] on tomato against late blight disease and demonstrated major antagonism. In addition, biocontrol of fungal disease Fusarium Head Blight (FHB) caused by F. graminearum on wheat heads in growth chamber conditions was studied using strain Pseudomonas chlororaphis G05 co-treated with: (i) wild-type strain G05; (ii) pliz-deleted mutant G05Δpliz; and (iii) mutant G05Δprn. The experiment showed wheat heads were infected with F. graminearum at rates of 5–8% and 80–90%, respectively, when co-sprayed with wild-type strain G05 and mutant G05Δprn [144], and PRN of wild type strain was found to be vigorously active against FHB disease.

The glasshouse experiments with talc-based formulation of S. marcescens ETR17 were similar to in vitro studies. Incidence of root rot in bacteria treated tea plants were considerably lower in comparison to untreated control as well as the fungicide treated sets. Additionally, ETR17 formulation
also increased the root and shoot length of the tea seedlings under both sterile and unsterile soil conditions in comparison to the untreated controls [85].

4.3. Pharmaceutical Applications

Pyrrolnitrin demonstrated strong protecting activity against various pathogenic fungi, especially against dermatophytosis [149]. It has been recommended for the treatment of superficial fungal infection of dermatophytic Trichophyton in Japan [150,151]. A patent has been granted on antifungal composition containing pyrrolnitrin and antymycotic imidazole compound in 1987 [152]. The product was commercialized under trade name Pyro-Ace W powder Spray by Fujisawa Pharmaceutical Company Ltd., Osaka. This was marketed by Pharmacia in Italy as “Micuritri” and, in combination with betamethasone valerate, it was formulated as “Beta Micurin” for athlete’s foot and ring worm diseases. The derivative, 3-cyanopyrroles, is more biologically active as pyrrolnitrin and very stable under light [153]. Jespers and co-workers (1993) reported a Fenpiclonil (CGA 142705) with more cytotoxicity for the representatives of Ascomycetes, Basidiomycetes, and Deuteromycetes. PRN formulated with carboxymethyl cellulose (5%) was injected intraperitoneally into mice [154] and LD50 was observed at a dosage of 500 mg Kg−1 [38].

In pharmacology, in vitro radioactive studies of pyrrolnitrin reflected that pyrrole ring is readily oxidized by enzymes undetected in urine and bile after administration [96]. Along with this, surface antigens of Candida albicans were released after treatment with PRN [38]. It also showed cytotoxicity at 10 µg mL−1 after 24 h and highest after 72 h on rat clonal pancreatic β-lines, INS-1. Thus, the compound becomes diabetogenic but appears nontoxic and insulinotropic at lower concentration [146]. PRN affected physiology of Caenorhabditis elegans, acted as repellent for adult nematodes to lower egg hatching by almost <50% at higher concentrations of PRN (1, 5, and 10 µg mL−1) after 24 h of exposure [84].

5. Conclusions

Natural bioactive PRN from different subgroups of rhizobacterial species display an array of biological properties, most prominently being the antifungal activity. Besides the leads on the formulation development and commercialization of the products for human and plant disease management, there exists tremendous scope with this small molecule for future research on making prominent functional derivatives with unmatched biological properties. The knowledge about metabolic route for biosynthesis, network of genes and enzymes linked with the intermediates, optimization of process parameters, assessment of efficient producer strains and optimized nutrient requirements of microbial species for improved PRN production need further improvement. We systematically rationalized chemistry and biological applications of PRN. However, the search for hypersecretory bacterial strains from the rhizosphere and soil habitat for economic production is being realized for maximum optimization of productivity of the molecule. Microbial systems tolerant to a wide range of organic solvents of industrial use might be a new route to economic PRN biosynthesis. Application of halogenase from high yielding bacteria could help to overcome issues of regioselectivity, dependency on chemical synthetic route and low yield of PRN. Besides, organic solvent tolerant halogenases for tailor-made synthesis and simplified downstream operations possible for PRN and green synthesis routes could also support industrial processes for PRN production.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used “conceptualization, X.X. and Y.Y.; methodology, X.X.; software, X.X.; validation, X.X., Y.Y. and Z.Z.; formal analysis, X.X.; investigation, X.X.; resources, X.X.; data curation, X.X.; writing—original draft preparation, X.X.; writing—review and editing, X.X.; visualization, X.X.; supervision, X.X.; project administration, X.X.; funding acquisition, Y.Y.”, please turn to the CRediT taxonomy for the term explanation. Authorship must be limited to those who have contributed substantially to the work reported. SP and AC prepared the MS. DPS has edited and reviewed the MS. RS has discussed biosynthetic pathways for PRN production. RP has contributed in shaping the MS in journal’s style and referencing pattern.
Acknowledgments: In this section you can acknowledge any support given which is not covered by the author contribution or funding sections. This may include administrative and technical support, or donations in kind (e.g., materials used for experiments). DPS and RS are thankful to Indian Council of Agricultural Research, India for institutional support. RP is thankful to Department of Science and Technology, Government of India for WOS-B project funding.

Conflicts of Interest: Declare conflicts of interest or state “The authors declare no conflict of interest.” Authors must identify and declare any personal circumstances or interest that may be perceived as inappropriately influencing the representation or interpretation of reported research results. Any role of the funders in the design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript, or in the decision to publish the results must be declared in this section. If there is no role, please state “The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results”. The authors declare no Conflict of interest.

References

1. Challinor, V.L.; Bode, H.B. Bioactive natural products from novel microbial sources. *Ann. N. Y. Acad. Sci.* 2015, 1354, 82–97. [CrossRef] [PubMed]
2. Hoffmann, T.; Krug, D.; Bozkurt, N.; Duddela, S.; Jansen, R.; Garcia, R.; Gerth, K.; Steinmetz, H.; Müller, R. Correlating chemical diversity with taxonomic distance for discovery of natural products in myxobacteria. *Nat. Commun.* 2018, 9, 803. [CrossRef] [PubMed]
3. Katz, L.; Baltz, R.H. Natural product discovery: Past, present, and future. *J. Ind. Microbiol. Biotechnol.* 2016, 43, 155–176. [CrossRef] [PubMed]
4. Williams, D.H.; Stone, M.J.; Hauck, P.R.; Rahman, S.K. Why are secondary metabolites (natural products) biosynthesized? *J. Nat. Prod.* 1989, 52, 1189–1208. [CrossRef] [PubMed]
5. Dixon, R.A. Natural products and plant disease resistance. *Nature* 2001, 411, 843–847. [CrossRef] [PubMed]
6. Kliebenstein, D.J. Secondary metabolites and plant/environment interactions: A view through *Arabidopsis thaliana* tinged glasses. *Plant Cell Environ.* 2004, 27, 675–684. [CrossRef]
7. Cragg, G.M.; Newman, D.J. Natural products: A continuing source of novel drug leads. *Biochim. Biophys. Acta* 2013, 1830, 3670–3695. [CrossRef]
8. Singh, S.B.; Pelea, F. Biodiversity, chemical diversity and drug discovery. In *Natural Compounds as Drugs Volume I*; Birkhäuser: Basel, Switzerland, 2008; pp. 141–174.
9. Hochberg, M.E. An ecosystem framework for understanding and treating disease. *Evol. Med. Public Health* 2018, 2018, 270–286. [CrossRef]
10. Hoddle, M.S.; Warner, K.; Steggall, J.; Jetter, K.M. Classical biological control of invasive legacy crop pests: New technologies offer opportunities to revisit old pest problems in perennial tree crops. *Insects* 2015, 6, 13–37. [CrossRef]
11. Gibson, D.M.; Donzelli, B.G.G.; Krasnoff, S.B.; Keyhani, N.O. Discovering the secondary metabolite potential encoded within entomopathogenic fungi. *Nat. Prod. Rep.* 2018, 2014, 1287–1305. [CrossRef]
12. Schmidt, C. Living in a microbial world. *Nat. Biotechnol.* 2017, 35, 401–403. [CrossRef] [PubMed]
13. Choudoir, M.J.; Pepe-Ranney, C.; Buckley, D.H. Diversification of secondary metabolite biosynthetic gene clusters coincides with lineage divergence in *Streptomyces*. *Antibiotics* 2018, 7, 12. [CrossRef] [PubMed]
14. Pyne, M.E.; Narcross, L.; Martin, V.J. Engineering plant secondary metabolism in microbial systems. *Plant Physiol.* 2019, 179, 844–861. [CrossRef] [PubMed]
15. Demain, A.L.; Fang, A. The natural functions of secondary metabolites. In *History of Modern Biotechnology*; Springer: Berlin/Heidelberg, Germany, 2000; pp. 1–39.
16. Gelband, H.; Molly Miller, P.; Pant, S.; Gandra, S.; Levinson, J.; Barter, D.; White, A.; Laxminarayan, R. The state of the world’s antibiotics 2015. *Wound Heal. South. Afr.* 2015, 8, 30–34.
17. Van Boeckel, T.P.; Brower, C.; Gilbert, M.; Grenfell, B.T.; Levin, S.A.; Robinson, T.P.; Teillant, A.; Laxminarayan, R. Global trends in antimicrobial use in food animals. *Proc. Natl. Acad. Sci. USA* 2015, 112, 5649–5654. [CrossRef] [PubMed]
18. Van Pee, K.H. Microbial biosynthesis of halometabolites. *Arch. Microbiol.* 2001, 175, 250–258. [CrossRef] [PubMed]
19. Gribble, G.W. Biological activity of recently discovered halogenated marine natural products. *Mar. Drugs* 2015, 13, 4044–4136. [CrossRef] [PubMed]
20. Field, J.A. Natural production of organohalide compounds in the environment. In *Organohalide-Respiring Bacteria*; Springer: Berlin/Heidelberg, Germany, 2016; pp. 7–29.
21. Marumo, S.; Abe, H.; Hattori, H.; Munakata, K. Isolation of a novel auxin, methyl 4-chloroindoleacetate from immature seeds of *Pisum sativum*. *Agric. Biol. Chem.* 1968, 32, 117–118. [CrossRef]
22. Engvild, K.C.; Egsgaard, H.; Larsen, E. Determination of 4-chloroindoleacetic acid methyl ester in *Vicieae* species by gas chromatography-mass spectrometry. *Physiol. Plant.* 1981, 53, 79–81. [CrossRef]
23. Gribble, G.W. The natural production of chlorinated compounds. *Environ. Sci. Technol.* 1994, 28, 310A–319A. [CrossRef]
24. Kuo, R.Y.; Chang, F.R.; Chen, C.Y.; Teng, C.M.; Yen, H.F.; Wu, Y.C. Antiplatelet activity of N-methoxycarbonyl aporphines from *Rollinia mucosa*. *Phytochemistry* 2001, 57, 421–425. [CrossRef]
25. Takahashi, Y.; Daitoh, M.; Suzuki, M.; Abe, T.; Masuda, M. Halogenated metabolites from the new Okinawan red alga *Laurencia yonagunienensis*. *J. Nat. Prod.* 2002, 65, 395–398. [CrossRef] [PubMed]
26. Gribble, G.W. Amazing organohalogens: Although best known as synthetic toxicants, thousands of halogen compounds are, in fact, part of our natural environment. *Am. Sci.* 2004, 92, 342–349. [CrossRef]
27. Hunt, S. Halogenated tyrosine derivatives in invertebrate scleroproteins: Isolation and identification. In *Methods in Enzymology*; Academic Press: Cambridge, MA, USA, 1984; Volume 107, pp. 413–438. [CrossRef]
28. Dème, D.; Fimiani, E.; Pommier, J.; Nunez, J. Free diiodotyrosine effects on protein iodination and thyroid hormone synthesis catalyzed by thyroid peroxidase. *Eur. J. Biochem.* 1975, 51, 329–336. [CrossRef] [PubMed]
29. Spande, T.F.; Garraffo, H.M.; Edwards, M.W.; Yeh, H.J.; Pannell, L.; Daly, J.W. Epibatidine: A novel (chloropyridyl) azabicycloheptane with potent analgesic activity from an Ecuadorian poison frog. *J. Am. Chem. Soc.* 1992, 114, 3475–3478. [CrossRef]
30. Van Pee, K.H. Biosynthesis of halogenated metabolites by bacteria. *Annu. Rev. Microbiol.* 1996, 50, 375–399. [CrossRef] [PubMed]
31. Gribble, G.W. The diversity of naturally produced organohalogenes. *Chemosphere* 2003, 52, 289–297. [CrossRef]
32. Fitch, R.W.; Spande, T.F.; Garraffo, H.M.; Yeh, H.J.; Daly, J.W. Phantasmidine: An epibatidine congener from the Ecuadorian poison frog *Epipedobates anthonyi*. *J. Nat. Prod.* 2010, 73, 331–337. [CrossRef] [PubMed]
33. Ehrlich, J.; Bartz, Q.R.; Smith, R.M.; Joslyn, D.A. Chloromyeetin, a new antibiotic from a soil actinomycete. *Science (Washington)* 1947, 147, 116. [CrossRef]
34. Duggar, B.M. Aureomycin: A product of the continuing search for new antibiotics. *Ann. N. Y. Acad. Sci.* 1948, 51, 177–181. [CrossRef]
35. Grove, J.F.; MacMillan, J.; Mulholland, T.P.C.; Rogers, M.T. 762. Griseofulvin. Part IV. Structure. *J. Chem. Soc.* (Resumed) 1952, 3977–3987. [CrossRef]
36. Takeda, R. Structure of a new antibiotic, pyoluteorin. *Ann. N. Y. Acad. Sci.* 1958, 91, 417. [CrossRef]
37. Oelrichs, P.B.; McEwan, T. Isolation of the toxic principle in *Acacia georginae*. *Nature* 1961, 190, 808–809. [CrossRef] [PubMed]
38. Arima, K.; Imanaka, H.; Kousaka, M.; Fukuta, A.; Tamura, G. Pyrrolnitrin, a new antibiotic substance produced by *Pseudomonas*. *Agric. Biol. Chem.* 1964, 28, 575–576. [CrossRef]
39. Morton, G.O.; Lancaster, J.E.; Van Lear, G.E.; Fulmor, W.; Meyer, W.E. Structure of nucleocidin. III. Revised structure. *J. Am. Chem. Soc.* 1969, 91, 1535–1537. [CrossRef] [PubMed]
40. Perkins, M.N.; Stone, T.W. An iontophoretic investigation of the actions of convulsant kynurenines and their interaction with the endogenous excitant quinolinic acid. *Brain Res.* 1982, 247, 184–187. [CrossRef]
41. Tunac, J.B.; Underhill, M. 2’-Chloropentostatin: Discovery, fermentation and biological activity. *J. Antibiot.* 1985, 38, 1344–1349. [CrossRef]
42. Shiomi, K.; Kakehashi, Y.; Yamanaka, H.; Kikuchi, T. Identification of arsenobetaine and a tetramethylarsonium salt in the clam *Meretrix lisora*. *Appl. Organomet. Chem.* 1987, 1, 177–183. [CrossRef]
43. Lee, M.D.; Manning, J.K.; Williams, D.R.; Kuck, N.A.; Testa, R.T.; Borders, D.B. Calicheamicins, a novel family of antitumor antibiotics. *J. Antibiot.* 1989, 42, 1070–1087. [CrossRef]
44. Zehner, S. Molekulargenetische und biochemische Untersuchungen zur Tryptophan-5-Halogenase aus der Biosynthese von Pyrroindomyein B in *Streptomyces sporeus* 2003, Dissertation. Available online: http://www.qucosa.de/fileadmin/data/qucosa/documents/1143/1084445344609-4611.pdf (accessed on 12 July 2019).
45. Hanefeld, U.; Floss, H.G.; Laatsch, H. Biosynthesis of the marine antibiotic pentabromopseudilin. Part 1. The benzene ring. *J. Org. Chem.* 1994, 59, 3604–3608. [CrossRef]
46. Trimurtulu, G.; Ohtani, I.; Patterson, G.M.; Moore, R.E.; Corbett, T.H.; Valeriote, F.A.; Demchik, L. Total structures of cryptophycins, potent antitumor depsipeptides from the blue-green alga Nostoc sp. strain GSV 224. J. Am. Chem. Soc. 1994, 116, 4729–4737. [CrossRef]

47. Lang, M.; Spitteler, P.; Hellwig, V.; Steglich, W. Stephanosporin, a “Traceless” Precursor of 2-Chloro-4-nitrophenol in the Gasteromycte stephanospora caroticolor. Angew. Chem. Int. Ed. 2001, 40, 1704–1705. [CrossRef]

48. Nelson, M.L.; Levy, S.B. The history of the tetracyclines. Ann. N. Y. Acad. Sci. 2011, 1241, 17–32. [CrossRef] [PubMed]

49. Gkotsi, D.S.; Dhaliwal, J.; McLachlan, M.M.; Mulholand, K.R.; Goss, R.J. Halogenases: Powerful tools for biocatalysis (mechanisms applications and scope). Curr. Opin. Chem. Biol. 2018, 43, 119–126. [CrossRef] [PubMed]

50. Mahoney, K.P.; Smith, D.R.; Bogosyan, E.J.; Goss, R.J. Access to high value natural and unnatural products through hyphenating chemical synthesis and biosynthesis. Synthesis 2014, 46, 2122–232. [CrossRef]

51. Murphy, C.D. New frontiers in biological halogenation. J. Appl. Microbiol. 2003, 94, 539–548. [CrossRef] [PubMed]

52. Smith, D.R.; Gruschow, S.; Goss, R.J. Scope and potential of halogenases in biosynthetic applications. Curr. Opin. Chem. Biol. 2013, 17, 276–283. [CrossRef] [PubMed]

53. Lively, D.H.; Gorman, M.; Haney, M.E.; Mabe, J.A. Metabolism of tryptophans by Pseudomonas aureofaciens. I. Biosynthesis of pyrrolnitrin. Antimicrob. Agents Chemother. 1966, 4, 462. [PubMed]

54. Nakano, H.; Umio, S.; Kariyone, K.; Tanaka, K.; Noguchi, H.; Ueda, I.; Nakamura, H.; Morimoto, T. Total synthesis of pyrrolnitrin, a new antibiotic. Tetrahedron Lett. 1966, 7, 737–740. [CrossRef]

55. Hamill, R.; Elander, R.; Mabe, J.; Gorman, M. Metabolism of tryptophans by Pseudomonas aureofaciens. V. Conversion of pyrrolnitrin to pyrrol. Antimicrob. Agents Chemother. 1967, 7, 388–396. [PubMed]

56. Hamill, R.L.; Elander, R.P.; Mabe, J.A.; Gorman, M. Metabolism of tryptophan by Pseudomonas aureofaciens III. Production of substituted pyrrolnitrins from tryptophan analogues. Appl. Microbiol. 1970, 19, 721–725. [PubMed]

57. Van Pee, K.H.; Ligon, J.M. Biosynthesis of pyrrolnitrin and other phenylpyrrole derivatives by bacteria. Nat. Prod. Rep. 2000, 17, 157–164. [CrossRef] [PubMed]

58. Van Pee, K.H.; Salcher, O.; Fischer, P.; Bokel, M.; Lingens, F. The biosynthesis of brominated pyrrolnitrin derivatives by Pseudomonas aureofaciens. J. Antibiot. 1993, 36, 1735–1742. [PubMed]

59. Burkhead, K.D.; Schisler, D.A.; Slininger, P.J. Pyrrolnitrin production by biological control agent Pseudomonas cepacia B37w in culture and in colonized wounds of potatoes. Appl. Environ. Microbiol. 1994, 60, 2031–2039. [PubMed]

60. Tichonov, M.S.; Boger, D.L. Yatakeycin: Total synthesis, DNA alkylation, and biological properties. Nat. Prod. Rep. 2008, 25, 220–226. [CrossRef] [PubMed]

61. Gosteli, J. Eine neue Synthese des Antibioticums Pyrrolnitrin. Helv. Chim. Acta 1972, 55, 451–460. [CrossRef] [PubMed]

62. Morrison, M.D.; Hanthorn, J.J.; Pratt, D.A. Synthesis of pyrrolnitrin and related halogenated phenylpyroles. Org. Lett. 2009, 11, 1051–1054. [CrossRef] [PubMed]

63. Alvarez, A.; Guzman, A.; Ruiz, A.; Velarde, E.; Muchowski, J.M. Synthesis of 3-arylpurroles and 3-pyrrolylacetylenes by palladium-catalyzed coupling reactions. J. Org. Chem. 1992, 57, 1653–1656. [CrossRef]

64. Elander, R.P.; Mabe, J.A.; Hamill, R.H.; Gorman, M. Metabolism of tryptophans by Pseudomonas aureofaciens VI. Production of pyrrolnitrin by selected Pseudomonas species. Appl. Microbiol. 1968, 16, 753–758. [PubMed]

65. Cherin, L.; Brandis, A.; Ismailov, Z.; Chet, I. Pyrrolnitrin production by an Enterobacter agglomerans strain with broad-spectrum activity toward fungal and bacterial phytopathogens. Curr. Microbiol. 1996, 32, 208–212. [CrossRef]

66. El-Banna, N.; Winkelmann, G. Pyrrolnitrin from Burkholderia cepacia: Antibiotic activity against fungi and novel activities against streptomycetes. J. Appl. Microbiol. 1998, 85, 69–78. [CrossRef] [PubMed]

67. Roberts, D.P.; McKenna, L.F.; Lakshman, D.K.; Meyer, S.L.F.; Kong, H.; de Souza, J.T.; Lydon, J.; Baker, C.J.; Buyer, J.S.; Chung, S. Suppression of damping-off of cucumber caused by Pythium ultimum with live cells and extracts of Serratia marcescens N4-5. Soil Biol. Biochem. 2007, 39, 2275–2288. [CrossRef]

68. Parry, R.; Nishino, S.; Spain, J. Naturally-occurring nitro compounds. Nat. Prod. Rep. 2011, 28, 152–167. [CrossRef] [PubMed]
69. Upadhyay, A.; Srivastava, S. Characterization of a new isolate of Serratia plymuthica with multiple mechanisms of antifungal activity provides biocontrol of Botrytis cinerea and Sclerotinia sclerotiorum diseases. Soil Biol. Biochem. 2003, 35, 323–331. [CrossRef]

70. Kalbe, C.; Marten, P.; Berg, G. Strains of the genus Serratia as beneficial rhizobacteria of oilseed rape with antifungal properties. Microbiol. Res. 1996, 151, 433–439. [CrossRef]

71. Ligon, J.M.; Hill, D.S.; Hammer, P.E.; Torkewitz, N.R.; Hofmann, D.; Kempf, H.J.; Pee, K.H.V. Natural strains of the genus Serratia as beneficial rhizobacteria of oilseed rape with an increased capacity for synthesis of pyrrolnitrin. J. Biotechnol. 2018, 211, 3–4. [CrossRef]

72. Kwak, Y.; Shin, J.H. Complete genome sequence of Burkholderia pyrocinia 2327T, the first industrial bacterium which produced antifungal antibiotic pyrrolnitrin. J. Biotechnol. 2015, 211, 3–4. [CrossRef]

73. Jung, B.K.; Hong, S.J.; Park, G.S.; Shin, J.H. Isolation of Burkholderia cepacia JBK9 with plant growth-promoting activity while producing pyrrolnitrin antagonistic to plant fungal diseases. Appl. Biol. Chem. 2018, 61, 173–180. [CrossRef]

74. Salcher, O.; Lingens, F. Isolation and characterization of a mutant of Burkholderia cepacia ATCC 15926 with an increased capacity for synthesis of pyrrolnitrin. Microbiology 1989, 118, 509–513. [CrossRef]

75. Salcher, O.; Lingens, F.; Fischer, P. Biosynthese von pyrrolnitrin nachweis von 4-(2-

76. Chang, C.J.; Floss, H.G.; Hook, D.J.; Mabe, J.A.; Manni, P.E.; Martin, L.L.; Schroder, K.; Shieh, T.L. Recent advances in biosynthesis of pyrrolnitrins by analogue-resistant Pseudomonas aureofaciens. J. Antibiot. 1981, 34, 555–566. [CrossRef] [PubMed]

77. Roitman, J.N.; Mahoney, N.E.; Janisiewicz, W.J.; Benson, M. A new chlorinated phenylpyrrole antibiotic produced by the antifungal bacterium Pseudomonas cepacia. J. Agric. Food Chem. 1990, 38, 538–541. [CrossRef] [PubMed]

78. Roitman, J.N.; Mahoney, N.E.; Janisiewicz, W.J.; Benson, M. Production and composition of phenylpyrrole metabolites produced by Pseudomonas cepacia. Appl. Microbiol. Biotechnol. 1990, 34, 381–386.

79. Takeda, F.; Janisiewicz, W.J.; Roitman, J.N.; Mahoney, N.E.; Janisiewicz, W.J.; Benson, M. Pyrrolnitrin delays postharvest fruit rot in strawberries. Hortic. Sci. 1990, 25, 320–322.

80. Hwang, J.; Chilton, W.S.; Benson, D.M. Pyrrolnitrin production by Burkholderia cepacia and biocontrol of Rhizoctonia stem rot of poinsettia. Biol. Control 2002, 25, 56–63. [CrossRef]

81. Upadhyay, A.; Srivastava, S. Characterization of a new isolate of Pseudomonas fluorescens strain Psd as a potential biocontrol agent. Lett. Appl. Microbiol. 2008, 47, 98–105. [CrossRef] [PubMed]

82. Park, J.Y.; Oh, S.A.; Anderson, A.J.; Neiswender, J.; Kim, J.C.; Kim, Y.C. Production of the antifungal compounds phenazine and pyrrolnitrin from Pseudomonas chlororaphis O6 is differentially regulated by glucose. Lett. Appl. Microbiol. 2011, 52, 532–537. [CrossRef] [PubMed]

83. Mujumdar, S.S.; Bashetti, S.P.; Chopade, B.A. Plasmid pUPI126-encoded pyrrolnitrin production by Acinetobacter haemolyticus A19 isolated from the rhizosphere of wheat. World J. Microbiol. Biotechnol. 2014, 30, 495–505. [CrossRef] [PubMed]

84. Nandi, M.; Selin, C.; Brassinga, A.K.C.; Belmonte, M.F.; Fernando, W.D.; deKievit, T.R. Pyrrolnitrin and hydrogen cyanide production by Pseudomonas chlororaphis strain PA23 exhibits nematicidal and repellent activity against Caenorhabditis elegans. PLoS ONE 2015, 10, e0123184. [CrossRef] [PubMed]

85. Purkayastha, G.D.; Mangar, P.; Saha, A.; Saha, D. Evaluation of the biocontrol efficacy of a Serratia marcescens strain indigenous to tea rhizosphere for the management of root rot disease in tea. PLoS ONE 2018, 13, e0191761. [CrossRef]

86. Martin, L.L.; Chang, C.J.; Floss, H.G.; Mabe, J.A.; Hagaman, E.W.; Wenkert, E. Carbon-13 nuclear magnetic resonance study on the biosynthesis of pyrrolnitrin from tryptophan by Pseudomonas. J. Am. Chem. Soc. 1972, 94, 8942–8944. [CrossRef]

87. Zhou, P.; Mocek, U.; Siesel, B.; Floss, H.G. Biosynthesis of pyrrolnitrin incorporation of 13C, 15N double-labelled D-and L-tryptophan. J. Basic Microbiol. 1992, 32, 209–214. [CrossRef] [PubMed]

88. Salcher, O.; Lingens, F.; Fischer, P. Biosynthese von pyrrolnitrin-nachweis von 4-(2′-amino-3′-chlorphenyl) pyrrol-2-carbonsäure. Tetrahedron Lett. 1978, 19, 3097–3100. [CrossRef] [PubMed]

89. FCtman, J.N.; Mahoney, N.E.; Janisiewicz, W.J. Production and composition of phenylpyrrole metabolites produced by Pseudomonas cepacia. Appl. Microbiol. Biotechnol. 1990, 34, 381–386.
90. Kirner, S.; Hammer, P.E.; Hill, D.S.; Altmann, A.; Fischer, I.; Weislo, L.J.; Lanahan, M.; van Pee, K.H.; Ligon, J.M. Functions encoded by pyrrolnitrin biosynthetic genes from Pseudomonas fluorescens. J. Bacteriol. 1998, 180, 1939–1943. [PubMed]

91. De Souza, J.T.; Raaijmakers, J.M. Polymorphisms within the prnD and pltC genes from pyrrolnitrin and pyoluteorin-producing Pseudomonas and Burkholderia spp. FEMS Microb. Ecol. 2003, 43, 21–34. [CrossRef] [PubMed]

92. Permeel, M.; Dhondt, L.; De Maeyer, K.; Adiobo, A.; Rabaey, K.; Hofte, M. Phenazines and biosurfactants interact in the biological control of soil-borne diseases caused by Pythium spp. Environ. Microbiol. 2008, 10, 778–788. [CrossRef] [PubMed]

93. Thongsri, Y.; Aromdee, C.; Yenjai, C.; Kanokmedhakul, S.; Chaiprasert, A.; Hamal, P.; Prariyachatigul, C. Detection of diketopiperazine and pyrrolnitrin, compounds with anti-Pythium insidiosum activity, in a Pseudomonas stutzeri environmental strain. Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech Repub 2014, 158, 378–383. [CrossRef] [PubMed]

94. Gerth, K.; Trowitzsch, W.; Wray, V.; Hofle, G.; IRSCHIK, H.; REICHENBACH, H. Pyrrolnitrin from Myxococcus fulus (myxobacteria). J. Antibiot. 1982, 35, 1101–1103. [CrossRef]

95. Sako, M.; Kihara, T.; Tanisaki, M.; Maki, Y.; Miyamae, A.; Azuma, T.; Kohda, S.; Masugi, T. Novel photodegradation of the antifungal antibiotic pyrrolnitrin in anhydrous and aqueous aprotic solvents. J. Org. Chem. 2002, 67, 668–673. [CrossRef] [PubMed]

96. Murphy, P.J.; Williams, T.L. Biological inactivation of pyrrolnitrin, Identification and synthesis of pyrrolnitrin metabolites. J. Med. Chem. 1972, 15, 137–139. [CrossRef]

97. Sultan, M.Z.; Park, K.; Lee, S.Y.; Park, J.K.; Varughese, T.; Moon, S.S. Novel oxidized derivatives of antifungal peptide from the bacterium Burkholderia cepacia K87. J. Antibiot. 2008, 61, 420–425. [CrossRef] [PubMed]

98. Liu, X.; Bimerew, M.; Ma, Y.; Muller, H.; Ovadis, M.; Eberl, L.; Berg, G.; Chernin, L. Quorum-sensing signaling is required for production of the antibiotic pyrrolnitrin in a rhizospheric biocontrol strain of Serratia plymuthica. FEMS Microbiol. Lett. 2007, 270, 299–305. [CrossRef] [PubMed]

99. van Pee, K.H.; Lingens, F. Detection of a bromoperoxidase in Streptomyces phaechochromogenes. FEBS Lett. 1984, 173, 5–8. [CrossRef]

100. Hill, D.S.; Stein, J.I.; Torkewitz, N.R.; Morse, A.M.; HOWELL, C.R.; Pachlatko, J.P.; Becker, J.O.; Ligon, J.M. Cloning of genes involved in the synthesis of pyrrolnitrin from Pseudomonas fluorescens and role of pyrrolnitrin synthesis in biological control of plant disease. Appl. Environ. Microbiol. 1994, 60, 78–85.

101. Kirner, S.; Krauss, S.; Sury, G.; Lam, S.T.; Ligon, J.M.; van Pee, K.H. The non-haem chloroperoxidase from Pseudomonas fluorescens and its relationship to pyrrolnitrin biosynthesis. Microbiology 1996, 142, 2129–2135. [CrossRef]

102. Bonsall, R.F.; Weller, D.M.; Thomashow, L.S. Quantification of 2,4-diacetylphloroglucinol produced by fluorescent Pseudomonas spp. in vitro and in the rhizosphere of wheat. Appl. Environ. Microbiol. 1997, 63, 951–955.

103. Brucker, R.M.; Baylor, C.M.; Walters, R.L.; Lauer, A.; Harris, R.N.; Minbiole, K.P. The identification of 2,4-diacetylphloroglucinol as an antifungal metabolite produced by cutaneous bacteria of the salamander Plethodon cinereus. J. Chem. Ecol. 2008, 34, 39–43. [CrossRef] [PubMed]

104. Selin, C.; Habibian, R.; Foritsanos, N.; Athukorala, S.N.; Fernando, D.; De Kievit, T.R. Phenazines are not essential for Pseudomonas chlororaphis PA23 biocontrol of Sclerotinia sclerotiorum, but do play a role in biofilm formation. FEMS Microbiol. Ecol. 2009, 71, 73–83. [CrossRef] [PubMed]

105. Morimoto, Y.; Hashimoto, M.; Hattori, K. The crystal structure of pyrrolnitrin. Tetrahedron Lett. 1968, 9, 209–211. [CrossRef]
10. Gorman, M.; Lively, D.H. Pyrrolnitrin: A new mode of tryptophan metabolism. In *Biosynthesis*; Springer: Berlin/Heidelberg, Germany, 1967; pp. 433–438.

11. Hammer, P.E.; Hill, D.S.; Lam, S.T.; van Pee, K.H.; Ligon, J.M. Four genes from *Pseudomonas fluorescens* that encode the biosynthesis of pyrrolnitrin. *Appl. Environ. Microbiol.* 1997, 63, 2147–2154. [PubMed]

12. Hubbard, B.K.; Walsh, C.T. Vancomycin assembly: nature’s way. *Angew. Chem. Int. Ed.* 2003, 42, 730–765. [CrossRef] [PubMed]

13. Dong, C.; Flecks, S.; Unversucht, S.; Haupt, C.; van Pee, K.H.; Naismith, J.H. Tryptophan 7-halogenase (prnA) structure suggests a mechanism for regioselective chlorination. *Science* 2005, 309, 2216–2219. [CrossRef] [PubMed]

14. Yeh, E.; Blasiak, L.C.; Koglin, A.; Drennan, C.L.; Walsh, C.T. Chlorination by a long-lived intermediate in the mechanism of flavin-dependent halogenases. *Biochemistry* 2007, 46, 1284–1292. [CrossRef] [PubMed]

15. van Pee, K.H.; Zehner, S. Enzymology and molecular genetics of biological halogenation. In *Natural Production of Organohalogen Compounds*; Springer: Berlin/Heidelberg, Germany, 2003; pp. 171–199.

16. Lee, J.; Simurdiak, M.; Zhao, H. Reconstitution and characterization of aminopyrrolnitrin oxygenase: A Rieske N-oxygenase that catalyzes unusual arylamine oxidation. *J. Biol. Chem.* 2005, 280, 36719–36728. [CrossRef] [PubMed]

17. Paulsen, I.T.; Press, C.M.; Ravel, J.; Kobayashi, D.Y.; Myers, G.S.; Mavrodi, D.V.; DeBoy, R.T.; Seshadri, R.; Ren, Q.; Madupu, R.; et al. Complete genome sequence of the plant commensal *Pseudomonas fluorescens* Pf-5. *Nat. Biotechnol.* 2005, 23, 873–878. [CrossRef] [PubMed]

18. Lee, J.K.; Zhao, H. Identification and characterization of the flavin: NADH reductase (PrnF) involved in a novel two-component arylamine oxygenase. *J. Bacteriol.* 2007, 189, 8556–8563. [CrossRef]

19. Ingelman-Sundberg, M.; Oscarson, M.; McLellan, R.A. Polymorphic human cytochrome P450 enzymes: An opportunity for individualized drug treatment. *Trends Pharmacol. Sci.* 1999, 20, 342–349. [CrossRef]

20. Kendrew, S.G.; Harding, S.E.; Hopwood, D.A.; Marsh, E.N.G. Identification of a flavin: NADH reductase subfamily. *J. Bacteriol.* 1995, 177, 5199–5205. [CrossRef] [PubMed]

21. Thibaut, D.; Ratet, N.; Bisch, D.; Faucher, D.; Debuysche, L.; Blanche, F. Purification of the two-enzyme system catalyzing the oxidation of the D-proline residue of pristinamycin IIB during the last step of pristinamycin IIA biosynthesis. *J. Bacteriol.* 1995, 177, 270, 17339–17343. [CrossRef] [PubMed]

22. Parry, R.J.; Li, W. An NADPH: FAD oxidoreductase from the valanimycin producer, *Streptomyces viridifaciens*. *Appl. Environ. Microbiol.* 2000, 66, 481–486. [CrossRef] [PubMed]

23. Duffner, F.M.; Muller, R. A novel phenol hydroxylase and catechol 2,3-dioxygenase from the thermophilic *Bacillus thermoleovorans* strain A2: Nucleotide sequence and analysis of the genes. *FEMS Microbiol. Lett.* 1998, 161, 37–45. [CrossRef]

24. Galan, B.; Diaz, E.; Prieto, M.A.; Garcia, J.L. Functional analysis of the small component of the 4-hydroxyphenylacetate 3-monooxygenase of *Escherichia coli* W: A prototype of a new flavin: NAD(P)H reductase subfamily. *J. Bacteriol.* 2000, 182, 627–636. [CrossRef] [PubMed]

25. Xun, L.; Sandvik, E.R. Characterization of 4-hydroxyphenylacetate 3-hydroxylase (HpA) of *Escherichia coli* as a reduced flavin adenine dinucleotide-utilizing monoxygenase. *Appl. Environ. Microbiol.* 2000, 66, 481–486. [CrossRef] [PubMed]

26. Kirchner, U.; Westphal, A.H.; Muller, R.; van Berkel, W.J. Phenol hydroxylase from *Bacillus thermoglucosidasius* A7 a two-protein component monoxygenase with a dual role for FAD. *J. Biol. Chem.* 2003, 278, 47545–47553. [CrossRef] [PubMed]

27. Umio, S.; Kariyone, K.; Tanaka, K.; Kishimoto, T.; Nakamura, H.; Nishida, M. Structure-activity studies of pyrrolnitrin analogues. *Chem. Pharm. Bull.* 1970, 18, 1414–1425. [CrossRef]

28. Warden, J.T.; Edwards, D.L. Electron spin resonance investigations of mitochondrial electron transport in *Neurospora crassa*: Characterization of paramagnetic intermediates in a standard strain. *Eur. J. Biochem.* 1976, 71, 411–418. [CrossRef]

29. Di Santo, R.; Costi, R.; Artico, M.; Massa, S.; Lampis, G.; Deidda, D.; Pompei, R. Pyrrolnitrin and related pyrroles endowed with antibacterial activities against *Mycobacterium tuberculosis*. *Bioorg. Med. Chem. Lett.* 1998, 8, 2931–2936. [CrossRef]

30. Raaaimakers, J.M.; Mazzola, M. Diversity and natural functions of antibiotics produced by beneficial and plant pathogenic bacteria. *Annu. Rev. Phytopathol.* 2012, 50, 403–424. [CrossRef] [PubMed]
131. Hori, Y.; Abe, Y.; Nakajima, H.; Takase, S.; Fujita, T.; Goto, T.; Okuhara, M.; Kohsaka, M. WB2838 [3-chloro-4-(2-amino-3-chlorophenyl)-pyrrole]: Non-steroidal androgen-receptor antagonist produced by a Pseudomonas. J. Antibiot. 1993, 46, 1327–1333. [CrossRef] [PubMed]

132. Fernando, W.D.; Nakkeeran, S.; de Kievit, T.; Porissanos, N.; Zhang, Y.; Paulit, T.C.; Li, Z.; Ramarathnam, R. Multiple mechanisms of biocontrol by Pseudomonas chlororaphis PA23 affect stem rot of canola caused by Sclerotinia sclerotiorum. In Proceedings of the 12th International Rapeseed Congress, Wuhan, China, 26–30 March 2007.

133. Lambowitz, A.M.; Slayman, C.W.; Slayman, C.L.; Bonner, W.D. Functional identification of the prnABC operon and its regulation in Serratia plymuthica. Appl. Microbiol. Biotechnol. 2018, 102, 3711–3721. [CrossRef] [PubMed]

134. Liu, X.; Yu, X.; Yang, Y.; Heeb, S.; Gao, S.; Chan, K.G.; Camara, M.; Gao, K. Production of the prnABCD operon and its regulation in Serratia plymuthica. Appl. Environ. Microbiol. 2009, 75, 1422–1437. [CrossRef] [PubMed]

135. Schmidt, S.; Blom, J.F.; Pernthaler, J.; Berg, G.; Baldwin, A.; Mahenthiralingam, E.; Eberl, L. Production of the antifungal compound pyrrolnitrin is quorum sensing-regulated in members of the Burkholderia cepacia complex. Environ. Microbiol. 2009, 11, 1422–1437. [CrossRef] [PubMed]

136. Gokdee, R.; Matthews, T.R. Evaluation of the in vitro and in vivo antifungal activity of pyrrolnitrin. Eval. In Vitro In Vivo Antifung. Act. Pyrrolnitrin 1968, 378–387.

137. Gordee, R.S.; Matthews, T.R. Systemic antifungal activity of pyrrolnitrin. Appl. Environ. Microbiol. 1969, 17, 690–694.

138. Nose, M.; Arima, K. On the mode of action of a new antifungal antibiotic, pyrrolnitrin. J. Antibiot. 1969, 22, 135–143. [CrossRef]

139. Tripathi, R.K.; Gottlieb, D. Mechanism of action of the antifungal antibiotic pyrrolnitrin. J. Bacteriol. 1969, 100, 318.

140. Howell, C.R.; Stipanovic, R.D. Suppression of Pythium ultimum-induced damping-off of cotton seedlings by Pseudomonas fluorescens and its antibiotic, pyoluteorin. Phytopathology 1980, 70, 712–715. [CrossRef]

141. Lambert, B.; Leyns, F.; van Rooyen, L.; Gossele, F.; Papon, Y.; Swings, J. Rhizobacteria of maize and their toxicological observations. J. Antibiot. 1993, 46, 378–387. [CrossRef] [PubMed]

142. Pfender, W.F.; Kraus, J.; Loper, J.E. A genomic region from Pseudomonas fluorescens and its antibiotic, pyoluteorin. In Synthesis and Chemistry of Agrochemicals III: ACS Symposium Series 649; American Chemical Society: Washington, DC, USA, 1992; pp. 395–404.

143. Watts, R.; Dahiya, J.; Chaudhary, K.; Tauro, P. Isolation and characterization of a new antifungal metabolite of Trichoderma reesei. Plant Soil 1988, 107, 81–84. [CrossRef]

144. Huang, R.; Feng, Z.; Chi, X.; Sun, X.; Lu, Y.; Zhang, B.; Lu, R.; Luo, W.; Wang, Y.; Miao, J.; et al. Pyrrolnitrin is more essential than phenazines for Trichoderma reesei of Pyrenophora tritici-repentis in wheat straw. Phytopathology 1993, 83, 1223–1228. [CrossRef]

145. Watkins, R.; Dahiya, J.; Chaudhary, K.; Tauro, P. Isolation and characterization of a new antifungal metabolite of Trichoderma reesei. Plant Soil 1988, 107, 81–84. [CrossRef]

146. Niis, R.B.; Russell, M.A.; Chrachri, A.; Moody, A.J.; Gilpin, M.L. Effects of the microbial secondary metabolites pyrrolnitrin, phenazine and patulin on INS-1 rat pancreatic β-cells. FEMS Immunol. Med. Microbiol. 2011, 63, 17–227. [CrossRef] [PubMed]

147. Janisiewicz, W.; Yourman, L.; Roitman, J.; Mahoney, N. Postharvest control of blue mold and gray mold of apples and pears by dip treatment with pyrrolnitrin, a metabolite of Pseudomonas cepacia. Plant Dis. 1991, 75, 490–494. [CrossRef]

148. Nyfeler, R.; Ackermann, P. Phenylpyrroles, a new class of agricultural fungicides related to the natural antibiotic pyrrolnitrin. In Synthesis and Chemistry of Agrochemicals III: ACS Symposium Series; Americal Chemical Society: Washington, DC, USA, 1992; pp. 395–404.

149. Kilani, J.; Fillinger, S. Phenylpyrroles: 30 years, two molecules and (nearly) no resistance. Front. Microbiol. 2016, 7, 2014. [CrossRef]

150. Nishida, M.; Matsubara, T.; Watanabe, N. Pyrrolnitrin, a new antifungal antibiotic, Microbiological and toxicological observations. J. Antibiot. 1965, 18, 211–219.
151. Tawara, S.; Matsumoto, S.; Hirose, T.; Matsumoto, Y.; Nakamoto, S.; Mitsuno, N.; Kamimura, T.; Yamaguchi, H. *In vitro* antifungal synergism between pyrrolnitrin and clotrimazole. *Jpn. J. Med. Mycol.* **1989**, *30*, 202–210. [CrossRef]

152. Umio, S.; Kamimura, T.; Kamishita, T.; Mine, Y. Antifungal composition employing pyrrolnitrin in combination with an imodazole compound. US patent US4636520A, 13 January 1987.

153. Nyfeler, R.; Ehrenfraund, J. Difluorobenzodioxyl cyanopyrrole microbicidal compositions. U.S. Patent 4925840A, 15 May 1990.

154. Jespers, A.B.; Davidse, L.C.; De Waard, M.A. Biochemical effects of the phenylpyrrole fungicide fenpiclonil in *Fusarium sulphureum* (Schlecht). *Pestic. Biochem. Physiol.* **1993**, *45*, 116–129. [CrossRef]

© 2019 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).