RESEARCH ARTICLE

Plant growth promoting endophyte Burkholderia contaminans NZ antagonizes phytopathogen Macrophomina phaseolina through melanin synthesis and pyrrolnitrin inhibition

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Abstract

The endophytic bacterium Burkholderia contaminans NZ was isolated from jute, which is an important fiber-producing plant. This bacterium exhibits significant growth promotion activity in in vivo pot experiments, and like other plant growth-promoting (PGP) bacteria fixes nitrogen, produces indole acetic acid (IAA), siderophore, and 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity. B. contaminans NZ is considered to exert a promising growth inhibitory effect on Macrophomina phaseolina, a phytopathogen responsible for infecting hundreds of crops worldwide. This study aimed to identify the possibility of B. contaminans NZ as a safe biocontrol agent and assess its effectiveness in suppressing phytopathogenic fungi, especially M. phaseolina. Co-culture of M. phaseolina with B. contaminans NZ on both solid and liquid media revealed appreciable growth suppression of M. phaseolina and its chromogenic aberration in liquid culture. Genome mining of B. contaminans NZ using NaPDoS and antiSMASH revealed gene clusters that displayed 100% similarity for cytotoxic and antifungal substances, such as pyrrolnitrin. GC-MS analysis of B. contaminans NZ culture extracts revealed various bioactive compounds, including catechol; 9,10-dihydro-12'-hydroxy-2'-methyl-5'-(phenylimethyl)- ergotaman 3',6',18-trione; 2,3-dihydro-3,5- dihydroxy-6-methyl-4H-pyran-4-one; 1-(1,6-Dioxooctadecyl)- pyrroline; 9-Octadecenamide; and 2-methoxy- phenol. These compounds reportedly exhibit tyrosinase inhibitory, antifungal, and antibiotic activities. Using a more targeted approach, an RP-HPLC purified fraction was analyzed by LC-MS, confirming the existence of pyrroline in the B. contaminans NZ extract. Secondary metabolites, such as catechol and ergotaman, have been predicted to inhibit melanin synthesis in M. phaseolina. Thus, B. contaminans NZ appears to inhibit
phytopathogens by apparently impairing melanin synthesis and other potential biochemical pathways, exhibiting considerable fungistatic activity.

Introduction

The continued use of expensive chemical fertilizers and fungicides have adversely affected human health and negatively impacted the environment; hence, the safe use of microorganisms that improve soil fertility, enhance plant growth and limit the growth of phytopathogenic fungi has been receiving immense attention from researchers [1]. Endophytes, mainly bacteria and fungi that spend at least a part of their lifespan within plant tissues without negatively affecting their hosts [2], have been intensely studied for years in terms of their diversity, metagenomics, combinatorial biosynthesis, plant growth promotion, biocontrol, bioremediation, etc. [3]. Plant-associated bacteria that aggressively colonize as symbiotic partners in the plant rhizosphere and roots with beneficial effects on plant growth are considered plant growth-promoting rhizobacteria (PGPR) [4]. In contrast, endophytic bacteria colonize the apoplasm or symplasm of the internal tissues of plants [5]. In general, with respect to growth promotion and protection against microbial infection, the beneficial effects of endophytes are considerably greater than those of several rhizobacteria [6].

The endophytes are grouped into three major clusters based on their different mechanisms of action. These contain: (i) biofertilization, which includes siderophore production, atmospheric nitrogen fixation, phosphate solubilization, and exopolysaccharides production; (ii) phyto-stimulation, which includes production of indole acetic acid, gibberellin, cytokinin, and ethylene; and (iii) phyto-biocontrol, which includes competing for iron, nutrients, and space; production of antibiotics, lytic enzymes, volatile compounds; and induction of systemic resistance [7]. Many studies have emphasized the ability of these microorganisms to promote and protect plant growth through additive/synergistic effects [1]; among them, Streptomyces spp. [8], B. subtilis [9], Pseudomonas parafulva, and Pantoea agglomerans [10] are a few examples.

Jute (Corchorus olitorius var. O-4) is an important natural fiber-producing cash crop in Southeast Asia [11]. Throughout its life cycle, jute is confronted by the destructive necrotrophic fungal pathogen Macrophomina phaseolina (Tassi) Goid., which causes charcoal rot disease in more than 500 plant species from approximately 100 families [12].

While screening for jute endophytes with potential inhibitory effects against M. phaseolina, we isolated an endophytic bacterium, Burkholderia contaminans NZ from the seed, which demonstrated promising antifungal activity [13]. This bacterium contained genes that are characteristic of PGPR, such as siderophore and ACC deaminase activities, phytohormone auxin (IAA) production, and nitrogen-fixing abilities, thereby promoting plant growth.

The genus Burkholderia, which belongs to the subphylum of β-proteobacteria, currently consists of 90 validly named species and several uncultivated candidate species [14]. They are ubiquitous organisms with high genetic versatility and adaptability, and are widespread in water, soil, plants, and animals, including humans [15].

However, the Burkholderia genus also comprises certain pathogenic species that threaten plant, animal, and human health [16]. Burkholderia contaminans is a member of the Burkholderia cepacia complex (Bcc) [17] which includes several closely related Burkholderia species. These opportunistic pathogens frequently colonize the lungs of cystic fibrosis (CF) and immune-compromised patients [18]. Furthermore, Bcc is the most frequently isolated clinical pathogen among Burkholderia spp., followed by B. mallei and B. pseudomallei [19]. Therefore,
The environmental release of *Burkholderia* species as a biocontrol agent in agriculture is severely debated due to difficulties in distinguishing plant growth-promoting and pathogenic bacteria [20]. Recent developments in *Burkholderia* taxonomy and molecular analysis may help in answering questions concerning differences between the pathogenic and ecological properties of *Burkholderia* species in an attempt to reconsider the possibility of using selected strains for biocontrol [21].

The present study aimed to determine the safety of *B. contaminans* NZ as a biocontrol agent and its effectiveness against phytopathogens, especially *M. phaseolina*. The entire genome sequence was analyzed in this study to unravel information regarding the safety of *B. contaminans* NZ as a PGPR and biocontrol agent based on their siderophores and secondary metabolites producing capacities, and the absence of virulence genes. In vitro data also revealed the attributes of growth promotion. Moreover, Gas chromatography–mass spectrometry (GC-MS) and liquid chromatography–mass spectrometry (LC-MS) analyses identified several potent antimicrobial compounds and antagonists of melanin synthesis, and the electron transport system produced by the bacterium in the presence of *M. phaseolina*. These compounds possibly antagonize fungal growth through different modes of action.

**Materials and methods**

**Bacterial, fungal strains and plant materials**

The phytopathogenic fungi *M. phaseolina* was obtained from the Bangladesh Jute Research Institute (BJRI), Dhaka. *Nigrospora sphaerica*, *Xylaria* spp., *Aspergillus fumigatus*, *Aspergillus niger*, and *Penicillium oxalicum* were collected from the Molecular Biology Lab, Department of Biochemistry and Molecular Biology, University of Dhaka. *Rhizoctonia solani* was obtained from the Bangladesh Agricultural University, Mymensingh, Bangladesh. All the fungi were grown and maintained on potato dextrose agar (PDA) and for GC-MS analysis, *M. phaseolina* was grown in potato dextrose broth (PDB) (HiMedia, India) at 28˚C. The antagonistic bacterial strain was isolated from jute seed as an endophytic bacterium as described by Coombs and Franco [22, 23] and identified as *B. contaminans* by 16S rRNA. Bacterial subculture was maintained on Tryptone Soy Agar (TSA) (HiMedia, India).

Jute seedlings were used to assess plant growth promotion activity of *B. contaminans* NZ. Fresh seeds of a jute variety (*Corchorus olitorius* var. O-4) were collected from BJRI. All tests were performed in triplicate if not mentioned otherwise.

**Whole genome sequencing of *B. contaminans* NZ**

*B. contaminans* NZ was cultured overnight in Tryptic Soy Broth (TSB) medium and incubated in an incubator shaker at 37˚C. The genomic DNA was extracted using the GenElute™ Bacterial Genomic DNA Kit (Sigma, Germany). A genomic library was constructed and employed for 300-bp paired-end whole-genome sequencing at the Genome Research Institute of North South University, Dhaka, Bangladesh using an IlluminaMiSeq platform (Illumina, San Diego, CA, USA) according to the manufacturer’s instructions. The generated raw reads (10xcoverage) were assembled using SPAdes version 3.11 [24]. The generated scaffolds were mapped and ordered using ABACAS—a reference-based assembler [25], considering *Burkholderia contaminans* CH1 as the reference genome [GenBank accession no. AP018357 to AP018360 (four entries)] [26].

**Genome sequence analysis and annotation.** For structural annotation of the genome Rapid Annotations using Subsystems Technology (RAST) server [27] was used (http://rast.nmpdr.org) along with SEED database for the functional annotation of predicted gene models. SEED also provides subsystem (collection of functionally related protein families) and derived
FIGfams (protein families) which represent the core of RAST annotation engine [28]. Subsequently, genome annotation through PIFAR annotation tool was used for the identification of bacterial genetic factors involved in plant host interaction [29]. Moreover, antiSMASH bacterial version 5.0 (https://antismash.secondarymetabolites.org/#!/start) [30] analysis was carried out to identify the biosynthetic gene clusters for secondary metabolites of *B. contaminans* NZ.

**Examining the presence of proteins fostering virulence and pathogenicity.** A total of 147 *Burkholderia* specific proteins reported for pathogenicity and virulence were collected from Virulence Factor Database (VFDB) (http://www.mgc.ac.cn/VFs/main.htm) [31] and investigated for the presence of virulence and pathogenicity related proteins in *B. contaminans* NZ with the help of BLASTp considering the e-value of at least 1e-10 and sequence identity, not less than 70%.

**Data deposition.** This Whole Genome Shotgun project has been deposited in DDBJ/ENA/GenBank under the accession QRBC00000000. The version described in this paper is version QRBC01000000.

**In vivo growth promotion study of *B. contaminans* NZ on jute**

**Inoculation of jute seeds with the endophyte.** At first *B. contaminans* NZ was cultured on TSA plates and then inoculated in TSB media and incubated at 37°C at a shaking speed of 180 rpm for 24–48 h. At the same time, scarification was done for jute seeds with sandpaper and the seeds surface was sterilized using 5% NaOCl. Sterilized seeds were dipped into *B. contaminans* NZ suspension. From McFarland standard, bacterial cells of 10⁷ CFU/ml were determined by a serial dilution method [32].

**In vivo pot experiment in hydroponic culture.** Jute seeds inoculated with a bacterial suspension were grown under controlled environmental conditions in a plant growth chamber (Weiss Technik India Private Limited) at 28°C, 70% relative humidity, and 16-h light/8-h dark cycle. The seeds were allowed to germinate for three days and later grown in a hydroponic culture system. 1.25 ml per litre of a modified Yoshida medium (NH₄NO₃ 91.4g, K₂SO₄ 71.4g, NaH₂PO₄.2H₂O 40.3g, CaCl₂.2H₂O 88.6g, and MgSO₄.7H₂O 324.0g per litre) and micronutrients (MnCl₂.4H₂O 1.5g, (NH₄)₆Mo₇O₂₄.4H₂O 0.074g, H₂BO₃ 0.934g, ZnSO₄.7H₂O 0.035 g, CuSO₄ .5H₂O 0.31g, FeCl₃ .6H₂O 7.70 g, citric acid 11.9 g and H₂SO₄ 50.0g per litre) [33] was added to the seedlings. pH of the solution was adjusted to 5.5 with NaOH. Thirty seeds were sown 1 cm deep in a cork sheet in separate pots. Jute plants were grown for 10 days in hydroponic culture and 5 plants were collected for the measurement of fresh and dry weights, shoot, and root lengths on 4, 7, and 10 days after transfer of the seedlings into the hydroponic solution. The experiment was repeated three times.

**Antifungal activity assay**

**Dual culture assay.** *In vitro* bacteria-fungal dual-culture assays were established by both agar well diffusion and cross streak methods in 9 cm diameter Petri dish systems containing PDA medium [34]. A 5 mm plug taken from the plate of an actively growing fungal colony was inoculated on one side of the Petri dish. Fresh cells of *B. contaminans* NZ were either streaked in 3 cm length parallel lines on the other side of the fungal plug or 20 μl of the overnight bacterial liquid culture was introduced into a 6 mm diameter well punched aseptically with a pipette tip in an agar plate. Control treatments containing only the fungus were also set up. The plates were incubated at 28°C for 4 to 5 days. The experiment was set up for four replicates. After incubation, the diameters of the fungal colonies were scored and measured.

Antifungal activity of *B. contaminans* NZ was determined against all the studied phytopathogenic fungi and the toxicity was expressed as a percentage of growth inhibition (PGI) and
calculated according to the Zygadlo et al. [35] formula,

\[
PGI(\%) = \frac{100 \times (GC - GT)}{GC}
\]

where GC represents the average diameter of the fungus grown on PDA (control); GT represents the average diameter of the fungus co-cultivated on the PDA dish with the antagonistic bacterium. A paired t-test was used to check whether the percentage of growth inhibition was significant.

**Co-culture of M. phaseolina and B. contaminans NZ.** For GC-MS analysis, *M. phaseolina* was cultured in 20 ml and 100 ml PDB media in two separate conical flasks. *B. contaminans* NZ was cultured in 80 ml and 100 ml of TSB media in two separate conical flasks.

On the second day of incubation, 20 ml *M. phaseolina* culture was mixed with 80 ml *B. contaminans* NZ to form the co-culture and put to incubation again. All the cultures were incubated at 28°C for 5 days for volatile compound extraction and observed for any phenotypic changes in *M. phaseolina* culture in the presence of *B. contaminans* NZ.

For HPLC and LC-MS analysis, a separate 1000 ml culture of *B. contaminans* NZ was cultured under similar conditions described above.

**Extraction of organic compounds from culture media**

On day 6 of single cultures of fungus, bacterium, and co-cultured media were filtered using Whatman filter paper and centrifuged at 4000 rpm for 7 minutes to remove cell debris. Supernatants were separated into individual conical flasks. 1:1 ethyl acetate (v/v) was added to each flask, shaken, and kept for 4 hours. The organic phases were separated and dried using a rotary evaporator. Finally, the extracts were dissolved in 2 ml of ethyl acetate. A separate extraction (for HPLC and LC-MS analysis) of *B. contaminans* NZ culture media was also conducted using n-hexane instead of ethyl acetate and shaken for 24 hrs. The extracted fraction was finally dissolved in methanol.

**GC-MS analysis of ethyl acetate extract.** Ethyl acetate extract of extracellular components was subjected to GC-MS analysis using Shimadzu GCMS-QP2010 Ultra; (Japan) mass detector connected with a capillary column of Rxi-5ms, 30 m long, 0.25 mm i.d.0.25 μm film thickness. 1μL of the sample was injected with splitless mode. Helium was used as the carrier gas with the flow rate was set at 1.0 ml/min. The column oven temperature was programmed from 40°C (1 min hold) at a rate of 10°C/min to 300°C (10 min hold). Injector temperature was maintained at 250°C. Temperatures of the mass interface and ion source were 250°C and 200°C respectively, and detector voltage was 0.4 KV. The analysis was carried out in the EI (electron impact) mode with 70e V of ionization energy. The analysis was performed in full scan mode, ranging from m/z 50 to 400. The compounds were detected after analyzing the mass spectrum of each component using the NIST11 library.

**HPLC and LC-MS analysis of methanolic extract.** Analytical HPLC Dionex Ultimate 3000 with a C18 column (Nucleodur, 250x4.6 mm, particle size 5μm, pore size 110Å, 100-5C18ec) was used to analyze the bacterial extract dissolved in methanol. After dissolution, the extract was filtered and 200 μl was injected into the column. A multi-step gradient system (solvent system: acetonitrile and water with 0.05% trifluoroacetic acid; flow rate: 1ml/min; temperature: 23°C) was performed with an optimized protocol (0–10 min, 10% acetonitrile; 10–30 min, 10–80% acetonitrile; 30–40 min, 80% acetonitrile; 40–50 min, 80–0% acetonitrile; volume fraction). The wavelength at 225 nm was used to detect compounds eluted from the column. Various fractions were collected containing high peak intensity in the chromatogram and the fractions were dried at low temperature in a lyophilizer (LYOVAC GT 2) and dissolved in methanol to test for antifungal activity and followed by a re-run in HPLC to confirm the reproducibility of the peak. The purified active fraction was then subjected to LC-MS.
For Liquid Chromatography (LC), Agilent 1290 Infinity II instrument was used. C18 column (2.1 mm x 100 mm x 1.8 um) (Zorbax RRHD Eclipse) was used as the stationary phase and 50:50 ratio of water (with 0.1% formic acid) and acetonitrile was used as the mobile phase on isocratic mode. The column temperature and the mobile phase flow rate were kept at 30˚C and 0.2 mL/min throughout the program. The sample injection volume was 5 ul. For Mass Spectrometry (MS) analysis, an Agilent 6420 Triple Quad Mass Spectrometer System was used, equipped with an electrospray ionization source operating at positive mode and scanning from m/z 100 to 1000. Nitrogen was used as the nebulizer gas, with pressure and flow set at 45 psi and 11 L/min, respectively. The capillary voltage was maintained at 4000 V, and dry gas temperature at 350˚C. Mass Hunter software was used to control and analyze the data. The compound was identified based on its MS fragmentation and analyzed according to literature [36].

**Identification of homologous melanin pathway(s) in M. phaseolina**

Fungi contain different pathways for melanin biosynthesis; among them two pathways namely 1,8-dihydroxynaphthalene (DHN)-melanin and L-3,4-dihydroxyphenylalanine (DOPA)-melanin pathways, are primarily found in the ascomycota group of fungi [37]. *M. phaseolina* proteins homologous to this pathway were searched using BLAST. Pathway proteins discovered through literature review and BLAST searches were tested against the *M. phaseolina* proteome. At least a 30% identity cut-off was taken to consider the homology between the target and query protein [38].

**Statistical analysis**

The average shoot length, average root length and average height from the three repeated experiment of *in vivo* pot experiment was taken for statistical analysis. Data obtained were subjected to analysis of variance (ANOVA) test against the control. The results presented as average means, standard deviation (SD), and standard error (SE) were determined by following the standard procedures.

**Results**

**Pot tests for *in vivo* assay of plant growth promotion**

*B. contaminans* NZ significantly increased the root and shoot lengths, and the average height of jute seedlings (P<0.05) (*Fig 1, S1 and S2 Tables*). The maximum root length of 11.28 cm, was 116.9% greater than that of the control. The effect on shoot length was also found to be significant; on average, it was 50.7% longer than that of the control jute plants. Fresh weight of 4-, 7-, and 10-day old jute seedlings increased by 141%, 122%, and 41%, respectively, compared to the controls (P<0.05).

In the presence of *B. contaminans* NZ, the dry weight of jute plants on an average, increased by 49.8% in all three replicates for each of the 4-, 7-, and 10-day measurements.

**Whole genome data analysis**

A summary of the whole genome annotation of *B. contaminans* NZ sequenced using Illumina MiSeq technology is presented in *S3 Table*. Functional analysis of *B. contaminans* NZ using the RAST server (Rapid Annotation using Subsystem Technology, http://rast.nmpdr.org), antiSMASH, and PIFAR (plant–bacteria information factor resource) revealed numerous genes and gene clusters (*Table 1, S4 Table*), which are reported to be exclusively associated with plant growth promotion.

As observed in other PGPR *Burkholderia* strains [39], the *nifHDK* operon required for nitrogen fixation was detected in *B. contaminans* NZ, along with the 1-aminocyclopropane-1-carboxylate (ACC) deaminase coding sequence, gene coding for iron(III) ABC transporter...
substrate-binding protein, phytoene synthase gene, the coding sequence for phosphotransferase system (PTS), and major facilitator superfamily (MFS) transporter genes. *In silico* analysis using RAST also revealed the presence of genes for indoleacetamide hydrolase involved in IAA biosynthesis.

**Fig 1. In vivo effect of plant growth promoting endophytic bacteria *B. contaminans* NZ.** (a) Bacterial effect significantly influences the increase in root and shoot lengths of jute seedlings when compared with the control jute plants at 4, 7, and 10 days. Comparisons of (b) shoot and root lengths, plant heights, and (c) fresh and dry weights of *B. contaminans* NZ-treated and control jute seeds. Error bars represent the standard error of the mean of the replicates. Shoot and root lengths are represented in cm, and fresh and dry weights in mg. Asterisk (*) denotes the difference between control and endophyte-treated samples at a significance level of *P* ≤ 0.05, as determined by ANOVA test. Values are mean(s) ± SD.

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### Table 1. List of genes involved in plant growth promotion activity detected from RAST, antiSMASH, PIFAR analysis of the *B. contaminans* NZ whole genome.

| Properties                        | Name                          | Biosynthetic genes           |
|-----------------------------------|-------------------------------|------------------------------|
| Phytohormone production and stress alleviation | Management of ethylene stress | ACC (1-Aminocyclopropane-1-carboxylic acid) deaminase |
|                                   | Production of IAA (Indole Acetic Acid) | Indole-3-glycerate phosphate synthase |
|                                   |                                | Indole pyruvate oxidoreductase |
|                                   |                                | Indole acetamide hydroxylate |
|                                   |                                | Tryptophan synthase |
|                                   |                                | Nitric hydratase |
| Phosphate solubilization          | pyrroloquinoline quinone gene  | *pqq* |
|                                  | Enolase                        | *eno* |
| Nitrogen fixation                 | *nif* gene cluster             | *nifHDK, nifQ,* |
|                                  | Others                         | *nodT, nir, nor, nolO* |
| Antibiotic biosynthesis           | Pyrrolnitrin                   | *prnA-prnD* |
| Siderophore Biosynthesis          | Polychelin                     | *pchR* |
|                                  | Ferric siderophore transport   | *pchD-pchA* |
|                                  | ABC-type siderophore export system | *feoB* |
|                                  | Siderophore pyoverdine         | *pvd* |

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production, which is a plant hormone associated with plant growth [40], and the *eno* gene, which assists in phosphate solubilization. Genome analysis also indicated the presence of pyrroloquinoline quinone synthase and glucose dehydrogenase, which are implicated in gluconic acid and 2-ketogluconic acid production, and required for mineral phosphate solubilization [41]. The annotated genome of *B. contaminans* NZ also contains 30 siderophore-related genes associated with ferric siderophore transport, ABC-type siderophore export system, arthrobac tin, siderophore pyoverdine, and an intact siderophore pyochelin biosynthesis *pch* gene cluster. From the PIFAR data, in *B. contaminans* NZ, the biosynthesis of the major siderophore pyochelin is apparently associated with five genes (PMID: 22261733) distributed over five different regions in the bacterial genome.

While examining the core biosynthesis genes, genetic loci related to antibiotic production were also found in *B. contaminans* NZ, as presented in Table 1. Moreover, genes for pyrrolnitrin, an antifungal secondary metabolite produced by certain Bcc and several other gram-negative bacteria, were also identified. This metabolite inhibits the growth of a wide range of fungi and pathogenic bacteria [42, 43].

**Most virulence genes absent in *B. contaminans* NZ**

Various virulence factors reported in pathogenic bacteria and other plant endophytic strains of *Burkholderia* (*B. contaminans* CH1, *B. contaminans* MS14) were screened for in the *B. contaminans* NZ genome, which failed in identifying major virulence-related genes, such as the biosynthesis genes for cable pili, toxoflavin, cepacian, and O-antigen (O-Ag) biosynthetic cluster (Table 2). This differs from pathogenic strains that contain many virulence genes. However, few virulent genes in *B. contaminans* NZ are present as incomplete gene clusters.

**Antifungal activity assay**

In dual culture, *B. contaminans* NZ substantially inhibited the growth of six plant fungal pathogens, namely Nigrospora sphaerica, Xylaria spp., Aspergillus fumigatus, Aspergillus niger, Penicillium oxalicum, and Rhizoctonia solani (Fig 2).

Table 2. List of major virulence related genes present or absent in *B. contaminans* NZ.

| Feature name                     | Relevant gene/gene cluster          | Status          |
|----------------------------------|-------------------------------------|-----------------|
| Actin based intracellular motility| *BimA*                              | Absent          |
| Adherence                        | *BoaA*                              | Absent          |
|                                  | *BoaB*                              | Absent          |
|                                  | Type VI pilin system                | Incomplete cluster (3 out of 11 genes) |
| Anti-phagocytosis (O antigens)   | Capsule I gene cluster              | Absent          |
| Secretion system                 | Bsa T3SS cluster                    | Absent          |
|                                  | T6SS-1 cluster                      | Absent          |
| Signaling                        | *Cdp*                               | Present         |
|                                  | Quorum sensing                      | Incomplete cluster (2 out of 8 genes) |
| Cepacian                         | *bceA-bceK, bceN-bceT*              | Absent          |
| Cable pilin gene                 | *cblA*                              | Absent          |
| Toxoflavin                       | *toxR, toxA-toxE*                   | Absent          |
| 2-heptyl-3-hydroxy-4(1H)-quinolone| *ppA-ppE*                           | Absent          |
| Hydrogencyanide                  | *hcnA-hcnC*                         | Absent          |
| Cu2+ and Zn2+ containing periplasmic SOD | *apaH-reG*                         | Present         |
| Zinc metalloprotease             | *Zmp*                               | Present         |

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In dual-culture assay, *B. contaminans* NZ demonstrated significant growth inhibitory activity, which ranged from 44% to 58% than that of the control (P<0.05), when co-cultured with all the tested fungi. The growth inhibition rates observed for each fungal species are shown in Table 3.

### Growth suppression and chromogenic aberration in *B. contaminans* NZ challenged *M. phaseolina*

*M. phaseolina* inoculated in liquid culture was initially pale in color, which assumes a characteristic black color within two to three days that perpetuates until day 5. However, a

| Fungal species              | Growth inhibition (%) (Mean ± SD) |
|----------------------------|-----------------------------------|
| *Xylaria* spp.             | 57.71 ± 1.88                      |
| *Aspergillus fumigatus*    | 45.94 ± 1.45                      |
| *Aspergillus niger*        | 54.38 ± 4.10                      |
| *Penicillium oxalicum*     | 55.64 ± 2.96                      |
| *M. phaseolina*            | 49.04 ± 3.41                      |
| *Nigrospora sphaerica*     | 52.99 ± 3.67                      |
| *Rhizoctonia solani*       | 44.31 ± 4.36                      |

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M. phaseolina culture challenged by B. contaminans NZ deviates from this usual behavior and retains the initial pale color up to day 5. Furthermore, M. phaseolina growth when subjected to co-culture with the bacterium remained practically static from day 2. Fig 3 shows the phenotypic changes in fungal growth from days 2 to 5.

Fig 3. Chromogenic aberration in B. contaminans NZ challenged M. phaseolina. (A) M. phaseolina, B. contaminans NZ, and their co-cultures on day 5. (B) M. phaseolina without B. contaminans NZ retains the black color (left) compared to its B. contaminans NZ challenged counterpart (right). Inhibition of M. phaseolina growth and its pigmentation in the presence of B. contaminans NZ is evident compared to the culture containing only M. phaseolina.

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Chemical analysis of bio-active compounds using GC-MS

GC coupled with MS was used to identify the organic compounds produced by the fungus, bacteria, and their co-culture, and to identify the chemical nature of the bioactive compounds responsible for inhibiting fungal growth. Three sets of secondary metabolites obtained from (i) *B. contaminans* NZ, (ii) *M. phaseolina*, and (iii) *B. contaminans NZ - M. phaseolina* dual culture aided in identifying more than 72 compounds. Among them, 9,10-dihydro-12'-hydroxy-2'-methyl-5'-(phenylmethyl) ergotaman 3',6',18-trione; 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one; 1-(1,6-dioxooctadecyl)- pyrrolidine; 9-octadecenamide; 3-trifluoroacetoxy-pentadecane;2-hexyldecanol; and catechol, reportedly exhibit various biological activities, including tyrosinase inhibition, antifungal, and antimicrobial effects (Table 4). For example, catechol acts as a suicide inhibitor of enzyme tyrosinase [44], which forms melanin in fungi. Furthermore, 9, 10-dihydro-12'-hydroxy-2'-methyl-5'-(phenylmethyl) ergotaman 3', 6', 18-trione acts as an antimicrobial and anti-inflammatory agent [45]. A partial list of compounds and their corresponding functions are provided in Table 4, and peaks from GC-MS analyses of *M. phaseolina* (M), *B. contaminans* NZ (B), and *M. phaseolina* and *B. contaminans* NZ co-culture (C) are shown in Fig 4.

Identification of pyrrolnitrin in methanolic extract of *B. contaminans* NZ culture. The chromatogram of RP-HPLC analysis of the methanolic crude extract showed various peaks (Fig 5A). RP-HPLC fractions were collected and their activity tested against *M. phaseolina*, wherein the fraction obtained at a retention time of 33.2 min exhibited antifungal activity (Fig 5B). The compound with a molecular ion peak of 257.1 (m/z) was identified as pyrrolnitrin via LC-MS analysis, after comparing with standard pyrrolnitrin (Fig 5C).

### Table 4. List of potential compounds with their biological activities and retention times found in GC-MS analysis of *B. contaminans* NZ (B), *M. phaseolina* (M), and their co-culture (C).

| Sl. No. | Compound Name                                | Culture Name | Retention Time (min) | Biological Activity                                      | Reference(s) |
|--------|----------------------------------------------|--------------|----------------------|----------------------------------------------------------|--------------|
| 1      | 2,5-Dimethyl pyrazine                        | B, C         | 6.28                 | Fungistatic activity                                      | [46]         |
| 2      | 2-Methoxy-phenol                             | C            | 9.30                 | Antioxidant, Cytotoxic                                   | [47]         |
| 3      | 5-Methyl-furfural                            | M, C         | 7.1                  | Antimicrobial, Antioxidant                               | [48]         |
| 4      | 2-Undecanethiol, 2-methyl                    | M, C, B      | 9.01/12.6            | Antimicrobial activity                                   | [49]         |
| 5      | 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one | B, C         | 10.4                 | Antifungal, Oxidative stress inducer                    | [50, 51]     |
| 6      | Catechol                                     | C            | 11.4                 | Antifungal activity, Suicide substrate inhibitors of tyrosinase | [44, 52, 53] |
| 7      | 1,4-Benzenediol / Hydroquinone               | B            | 11.42                | Cytotoxic activity, Protein kinase inhibition            | [54]         |
| 8      | 2-(1,1-Dimethylethyl)-4,6-dinitro-phenol      | B            | 12.8                 | Herbicide                                                | [55]         |
| 9      | Decahydro-1,4-dimethoxy naphthalene          | B            | 14.6                 | Antimicrobial activity                                   | [56]         |
| 10     | 2-Hexyldecanol                               | C            | 16.06                | Suppresses melanin synthesis                             | [57]         |
| 11     | 3-trifluoroacetoxypentadecane                | C            | 16.6                 | Antimicrobial activity                                   | [58]         |
| 12     | Methoxycetic acid, 3-tridecyl ester          | M, B         | 16.6/17.8            | Cytotoxicity                                             | [59]         |
| 13     | Pyrrolo[1,2-a]pyrazine-1,4-dione             | M, B         | 19.6/19.9            | Antioxidant agent, Antifungal activity, Antibiotic activity | [60–62]     |
| 14     | 3,6-Diisopropyliiperazin-2,5-dione            | B            | 20.1                 | Antimalarial activity                                    | [63]         |
| 15     | 1-(1,6-Dioxooctadecyl)-pyrrolidine           | B, C         | 20.23/21.2           | Antioxidant activity                                     | [64]         |
| 16     | n-Hexadecanoic acid                          | B, M, C      | 21.04                | Antibacterial, Antifungal, Cytotoxicity,                 | [65]         |
| 17     | Hexadecamethylheptasiloxane                  | B, C         | 23.8                 | Antifungal                                              | [66]         |
| 18     | 9-Octadecanamide                             | B, C         | 24.8                 | Antimicrobial, Anti-inflammatory                         | [58]         |
| 19     | 9,10-Dihydro-12'-hydroxy-2'-methyl-5'-(phenylmethyl)-, (5'alpha,10alpha) - ergotaman-3',6',18-trione | B, C | 24.9/25.1 | Antimicrobial, Anti-inflammatory, Alpha-amyalase inhibitory activity | [45] |
| 20     | Di-n-octyl phthalate                         | B, M         | 25.9/ 26.35          | Antifungal and antioxidant                              | [67, 68]     |
Identification of putative melanin pathways in *M. phaseolina* through homology-based search

After an extensive literature review of different melanin pathways active in fungi, polyketide synthase, tetrahydroxy naphthalene reductase, trihydroxy naphthalene reductase, scytalone

![Fig 4. Peaks from GC-MS analyses with potent volatile compounds. (A) *M. phaseolina*, (B) *M. phaseolina* and *B. contaminans* NZ co-culture, and (C) *B. contaminans* NZ. The number on different peaks corresponds to the serial number of the compounds in Table 4.](https://doi.org/10.1371/journal.pone.0257863.g004)
Dehydratase, and tyrosinase proteins were selected, and their corresponding homologs in *M. phaseolina* were identified through a BLAST search. The details are presented in Table 5. In addition, putative pathways proposed in S1 Fig presents the order in which the reactions occur during melanin synthesis in *M. phaseolina*.

**Discussion**

Since chemical control and most cultural practices are not effective representative tools that limit the pathogenic fungal growth and their distribution [69], the use of biocontrol agents has become a promising alternative in agriculture. Biocontrol using plant growth-promoting rhizobacteria (PGPR) is a potentially attractive and efficient disease management approach, as it...
promotes plant growth, enhances tolerance to abiotic stress [70], suppresses pathogens locally, and induces systemic resistance (ISR) against a broad range of crop diseases [71, 72]. Several studies conducted on endophytes have emphasized their ability in promoting plant growth and their additive/synergistic effects on plant growth and protection. In general, such growth-promoting rhizospheric bacteria belong to the following species: *Alcaligenes*, *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Klebsiella*, and *Pseudomonas* [8, 73]. The endophytic strain *B. contaminans* NZ isolated from jute has also been characterized in vivo for its potential plant growth-promoting (PGP) traits, which significantly increased both yield and biomass when jute seedlings were inoculated with the bacterium, and notably reduced the development of disease symptoms caused by *M. phaseolina*. As *B. contaminans* NZ exhibited growth promotion and apparent protection against the phytopathogen *M. phaseolina*, its whole genome was sequenced to determine the characteristic genetic features. Genome annotation of *B. contaminans* NZ assisted in identifying genes related to phytohormone production, stress alleviation, phosphate solubilization, nitrogen fixation, antibiotic biosynthesis, and siderophore biosynthesis. *In vitro* experiments of the endophyte also displayed significant production of the above-listed compounds (S5 Table, S2 Fig).

Certain *Burkholderia* species demonstrate clinical associations and plant-pathogenic traits [74, 75]; therefore, genes related to virulence and pathogenicity were also investigated in the *B. contaminans* NZ genome. Several bacterial species from the *B. cepacia* complex (Bcc) are opportunistic pathogens [76]. *B. cenocepacia* strains expressing both cable (Cbl) pili and 22-KDa adhesin proteins have been reported to bind strongly to cytokeratin 13 (CK13), which efficiently infects the host cell by invading the squamous epithelium [77]. However, the cblA (giant cable pilus) gene was absent in the symbiotic and legume-nodulating species [78]. *B. contaminans* NZ also lacks the cable pili biosynthesis gene cluster, indicating their inability to attach to the host cell, which is required to initiate infection. Furthermore, VgrG-5, which is a *Burkholderia* type VI secretion system 5-associated protein, required for complete mammalian virulence [79], is also absent in *B. contaminans* NZ genome. Virulence-associated protein sequences (for chemotaxis, attachment, type 3 and type 6 secretion systems; T3SS and T6SS) and typically described hallmark features representing the true and opportunistic pathogenic *Burkholderia* strains are also absent in *B. contaminans* NZ. Cystic fibrosis (CF)-related O-antigen of lipopolysaccharides associated with transmitting infections in CF patients [80] and zinc metalloproteases that may be involved in the overall virulence of several Bcc strains [81] are also absent. Among the virulence-associated proteins of *Burkholderia*, only the sodC gene encoding a Cu\(^{2+}\) and Zn\(^{2+}\) containing periplasmic superoxide dismutase that contributes in intracellular survival, which indicates self-protection ability in CF patients, is present in the genome [82]. *Burkholderia* species commonly produce plant-toxic secondary metabolites, polysaccharides, and other toxins such as rice grain rot and wilt causal agent, toxoflavin; exo-

Table 5. Homologous proteins of melanin pathways found in *M. phaseolina*.

| Query Accession | Query Name               | Subject Accession | Subject Name               | Percent Identity |
|-----------------|--------------------------|-------------------|---------------------------|------------------|
| K2S0W9          | Betaketocycl synthase     | D7RJP3            | Polyketed synthase        | 31.25            |
| K2RKI0          | Short-chain dehydrogenase/ reductase SDR | Q12634          | Tetrahydroxy naphthalene reductase | 75               |
| K2R7Y4          | Scytalone dehydratase     | W3XEE6            | Scytalone dehydratase     | 82.7             |
| K2R777          | Tyrosinase               | U7PL38            | Tyrosinase                | 31.132           |

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polysaccharide toxin, cepacian; hydrogen cyanide (HCN); and 2-heptyl-3-hydroxy-4(1H)-quinolone [80]. These pathogenic genes were absent in this jute endophytic bacterium.

The B. contaminans NZ genome also contained antibiotic-biosynthetic genes. Thus, to gather further evidence, M. phaseolina and several other phytopathogenic fungi, namely, Xylaria spp., Aspergillus fumigatus, Aspergillus niger, Penicillium oxalicum, Nigrospora sphaerica, and Rhizoctonia solani were co-cultured with B. contaminans NZ, where all fungi tested were suppressed with varying degrees of inhibition (Fig 2, Table 3), confirming its antifungal activity.

Growth inhibition of M. phaseolina by B. contaminans NZ caused marked morphological changes and chromogenic aberration in the phytofungus [13]. Such morphological changes in the cell membrane have also been reported for B. cepacia-mediated inhibition of F. solani and C. dematiurn [83]. Burkholderia CF66I noticeably alters R. solani hyphae with multiple branches and swelling [84]. In previous reports, co-culturing M. phaseolina and B. contaminans NZ in solid media caused the fungus to lose its characteristic black color, restricted its growth, and attenuated its infectivity [13]. The present study also observed a similar deviation in pigmentation and growth repression in liquid co-culture (Fig 3).

Various reports have demonstrated that secondary metabolites produced by certain bacteria can influence fungal growth [85, 86]. Therefore, secondary metabolites were analyzed to explain chromogenic aberration and growth suppression of M. phaseolina when co-cultured with B. contaminans NZ. GC-MS analysis of ethyl acetate extracts of culture supernatant revealed over 72 compounds, among which 20 compounds are biologically important; 2,5-dimethyl pyrazine, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, hexadecamethyloctasiloxane, 9-octadecenamide, and 9,10-dihydro-12'-hydroxy-2'-methyl-5'-(phenylmethyl)-, (5' alpha,10 alpha)- ergotaman-3',6',18'-trione compounds were discovered in both B. contaminans NZ and its co-cultures, but were absent in the M. phaseolina extract. 2,5-dimethyl pyrazine, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, and hexadecamethyloctasiloxane exhibits antifungal activities [46, 50, 51, 87]. In addition, 9-octadecenamide and 9,10-dihydro-12'-hydroxy-2'-methyl-5'-(phenylmethyl)-, (5' alpha,10 alpha)-ergotaman-3',6',18'-trione reportedly exhibits antimicrobial properties [58, 88]. The absence of these compounds in M. phaseolina extract implies that they are most likely produced only by B. contaminans NZ, thereby inhibiting the pathogen in the liquid media. 2-heptyldecanol, which suppresses melanin biosynthesis [57], and catechol, which demonstrates antifungal properties [52], are exclusively produced in the co-culture. Catechol, is also known as a suicide substrate for enzyme tyrosinase [53], which is involved in the DOPA-melanin biosynthesis pathway [37]. These compounds apparently produced only by B. contaminans NZ, thereby inhibiting the pathogen in the liquid media. 2-heptyldecanol, which suppresses melanin biosynthesis [57], and catechol, which demonstrates antifungal properties [52], are exclusively produced in the co-culture. Catechol, is also known as a suicide substrate for enzyme tyrosinase [53], which is involved in the DOPA-melanin biosynthesis pathway [37]. These compounds apparently produced only by B. contaminans NZ, thereby inhibiting the pathogen in the liquid media.

Burkholderia spp. reportedly produces potent antifungal compound pyrrolnitrin [89]. This compound exhibits inhibitory effects by obstructing the synthesis of vital biomolecules (DNA, RNA, and protein), uncoupling oxidative phosphorylation, impeding mitotic division, and inhibiting several biological mechanisms. The genome of B. contaminans NZ also contained a gene cluster for pyrrolnitrin synthesis (Table 2). Although the GC-MS data revealed the presence of several compounds that implied possible modes for M. phaseolina growth suppression, pyrrolnitrin in the secondary metabolite profile was absent. Therefore, the extraction process was altered by substituting the extraction solvent with n-hexane instead of ethyl acetate to
identify pyrrolnitrin, which is in agreement with earlier reports that employed a similar method [43]. Thereafter rest of the method remained similar except that the extract was dissolved in methanol instead of ethyl acetate. RP-HPLC was performed to purify the active compound(s) to detect the appreciable suppression of fungal growth by the crude extract. Only one fraction exhibited considerable inhibitory activity against \textit{M. phaseolina}, and its mass was identified using LC-MS analysis (257.1 Da), which was identical to the standard pyrrolnitrin (molecular weight: 257.07 g/mol).

A previous iTRAQ proteomic analysis of \textit{M. phaseolina} challenged with \textit{Burkholderia contaminans} showed the downregulation of beta-ketoacyl synthase, scytalone dehydratase, tyrosinase, enzymes of the DHN-melanin, and DOPA-melanin pathways [13]. Catechol present in the co-culture of \textit{B. contaminans} NZ and \textit{M. phaseolina} can explain the downregulation of tyrosinase. Kojic acid [5-hydroxy-2-((hydroxymethyl)-4H-pyran-4-one] is a prominent tyrosinase inhibitor [37], and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, identified by GC-MS analysis, is an oxidized form of kojic acid and is expected to act identical to its reduced counterpart. This derivatization could have occurred during sample processing for GC-MS analysis.

Further investigation of the \textit{M. phaseolina} enzymes involved in melanin synthesis, similar to those reported earlier in other fungi, led to the identification of SDR–short chain dehydrogenase/reductase, which is similar to both trihydroxy naphthalene reductase [90] and tetrahydroxy naphthalene reductase [91], which are enzymes of the DHN-melanin pathway [92]. Tyrosinase [92, 93] performs multiple catalysis in the DOPA-melanin pathway; it converts phenylalanine to tyrosine, tyrosine to DOPA, and DOPA to DOPA quinone, which is eventually converted to melanin further downstream. However, both DOPA-melanin and DHN-melanin pathways are yet to be fully elucidated. This reduces the scope of finding other homologous pathway proteins in \textit{M. phaseolina}. The percent identity of beta-ketoacyl synthase and tyrosinase was lower (~31%) than that of tetrahydroxy naphthalene reductase, trihydroxy naphthalene reductase, and scytalone dehydratase (~75% or more). This was possibly due to only few reports that describe the two genes with precise genomic features. Based on this evidence, a tentative melanin pathway was proposed for \textit{M. phaseolina} in this study (S1 Fig).

**Conclusion**

Whole genome analysis and secondary metabolite characterization have acknowledged \textit{B. contaminans} NZ as a good biocontrol agent, in addition to its role in plant growth promotion. It suppresses the growth of multiple fungi, especially the phytopathogen \textit{M. phaseolina}, by using catechol, pyrrolnitrin, and other antimicrobial agents. Compounds identified in the extracts of \textit{B. contaminans} NZ or the co-culture of \textit{B. contaminans} NZ and \textit{M. phaseolina} contain compounds that inhibit melanin biosynthesis, which possibly contributes to the observed chromogenic aberration and growth suppression of the fungi. \textit{B. contaminans} NZ can be established as a bio-control agent by conducting toxicity tests to ensure its safety, followed by field experiments to determine its efficacy under different environmental conditions. Hence, further studies are needed to optimize the formulation and application methods of \textit{B. contaminans} NZ to fully maximize its potential as an effective agent in controlling \textit{M. phaseolina}.

**Supporting information**

S1 Fig. Proposed melanin pathways in \textit{M. phaseolina} based on homology search. (TIF)
S2 Fig. Qualitative assay for (a) siderophore, (b) ACC deaminase, and (c) nitrogen of \textit{B. contaminans} NZ. (TIFF)

S1 Table. Average root length, shoot length, and plant height of bacteria treated jute seedlings vs. untreated control in a pot experiment at 4, 7, and 10 days. The data of three replicates per experiment are presented as means and standard deviations. (DOCX)

S2 Table. Fresh weight and dry weight of the bacteria treated jute seedlings vs. untreated control in a pot experiment at 4, 7, and 10 days. The data of three replicates per experiment are presented as mean(s) and standard deviation(s). (DOCX)

S3 Table. Genomic features of \textit{Burkholderia contaminans} NZ. (DOCX)

S4 Table. Location of genes involved in plant growth promotion activity detected from RAST, antiSMASH, and PIFAR analysis of the whole genome of \textit{B. contaminans} NZ. (DOCX)

S5 Table. \textit{In vitro} plant growth promotion attributes of \textit{B. contaminans} NZ. (DOCX)

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References

1. Adesemoye AO, Torbert HA, Kloeper JW. Plant growth-promoting rhizobacteria allow reduced application rates of chemical fertilizers. Microb Ecol. 2009; 58: 921–929. https://doi.org/10.1007/s00248-009-9531-y PMID: 1946478

2. Haroim PR, van Overbeek LS, Berg G, Pirttilä AM, Compart S, Campisano A, et al. The Hidden World within Plants: Ecological and Evolutionary Considerations for Defining Function of Microbial Endophytes. Microbiol Mol Biol Rev. 2015; 79: 293–320. https://doi.org/10.1128/MMBR.00050-14 PMID: 26136581

3. Kaul S, Sharma T, Dhar MK. “Omics” tools for better understanding the plant–endophyte interactions. Front Plant Sci. 2016; 7: 955. https://doi.org/10.3389/fpls.2016.00955 PMID: 27446181

4. Kloeper JW, Leong J, Teintze M, Schroth MN. Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. Nature. 1980; 286: 885–886. https://doi.org/10.1038/286885a0

5. Ma Y, Prasad MNV, Rajkumar M, Freitas H. Plant growth promoting rhizobacteria and endophytes accelerate phyto remediation of metaliferous soils. Biotechnology Advances. Biotechnol Adv; 2011. pp. 248–258. https://doi.org/10.1016/j.biotechadv.2010.12.001 PMID: 21147211

6. Pillay VK, Nowak J. Inoculum density, temperature, and genotype effects on in vitro growth promotion and epiphytic and endophytic colonization of tomato (Lycopersicon esculentum L.) seedlings inoculated with a pseudomonad bacterium. Can J Microbiol. 1997; 43: 354–361. https://doi.org/10.1139/m97-049

7. Mitter B, Petric A, Shin MW, Chain PSG, Hauberg-Lotte L, Reinhold-Hurek B, et al. Comparative genome analysis of Burkholderia phytofirmans PsJN reveals a wide spectrum of endophytic lifestyles based on interaction strategies with host plants. Front Plant Sci. 2013;4. https://doi.org/10.3389/fpls.2013.0004 PMID: 2335843

8. Vurukonda SSKP, Giovandari D, Stefan E. Plant growth promoting and biocontrol activity of streptomyces spp. As endophytes. International Journal of Molecular Sciences. MDPI AG; 2018. https://doi.org/10.3390/jm19040952 PMID: 29565834

9. Rajamanickam S, Kirthikeyan G, Kavino M, Manoranjitham SK. Biohardening of micropropagated banana using endophytic bacteria to induce plant growth promotion and restrain rhizome rot disease caused by Pectobacterium carotovorum subsp. carotovorum. Sci Hortic (Amsterdam). 2018; 231: 179–187. https://doi.org/10.1016/j.scienta.2017.12.037

10. Verma SK, Kingsley K, Bergen M, English C, Elmore M, Kharwar RN, et al. Bacterial endophytes from rice cut grass (Leersia oryzoides L.) increase growth, promote root gravitropic response, stimulate root hair formation, and protect rice seedlings from disease. Plant Soil. 2018; 422: 223–238. https://doi.org/10.1007/s11104-017-3339-1

11. Babu BK, Saxena AK, Srivastava AK, Arora DK. Identification and detection of Macrophomina phaseolina by using species-specific oligonucleotide primers and probe. Mycologia. 2007; 99: 797–803. https://doi.org/10.3852/mycologia.99.6.797 PMID: 18333503

12. Islam MS, Haque MS, Islam MM, Emdad EM, Halim A, Hossen QMM, et al. Tools to kill: Genome of one of the most destructive plant pathogenic fungi Macrophomina phaseolina. BMC Genomics. 2012; 13: 493. https://doi.org/10.1186/1471-2164-13-493 PMID: 22992219

13. Zaman NR, Kumar B, Nasrin Z, Islam MF, Maiti TK, Khan H. Proteome Analyses Reveal Macrophomina phaseolina’s Survival Tools When Challenged by Burkholderia contaminans NZ. ACS Omega. 2020; 5: 1352–1362. https://doi.org/10.1021/acsomega.9b01870 PMID: 32010805

14. Depoorter E, Bull MJ, Peeters C, Coenev T, Vandamme P, Mahenthiralingam E. Burkholderia: an update on taxonomy and biotechnological potential as antibiotic producers. Applied Microbiology and Biotechnology. Springer Verlag; 2016. pp. 5215–5229. https://doi.org/10.1007/s00253-016-7520-x PMID: 27115756

15. Mannaa M, Park I, Seo YS. Genomic features and insights into the taxonomy, virulence, and benevolence of plant-associated burkholderia species. International Journal of Molecular Sciences. MDPI AG; 2018. https://doi.org/10.3390/jm19040952 PMID: 30598000

16. Li W, Roberts DP, Dery PD, Meyer SLF, Lohrke S, Lumsden RD, et al. Broad spectrum anti-biotic activity and disease suppression by the potential biocontrol agent Burkholderia ambifaria BC-F. Crop Prot. 2002; 21: 129–135. https://doi.org/10.1016/S0261-2194(01)00074-6

17. Degrosi JJ, Merino C, Issamendia AM, Ibarra LM, Collins C, Bo NE, et al. Whole Genome Sequence Analysis of Burkholderia contaminans FFH2055 Strain Reveals the Presence of Putative β-
Lactamases. Curr Microbiol. 2019; 76: 485–494. https://doi.org/10.1007/s00284-019-01653-4 PMID: 30783798

18. Ghequire MGK, De Mot R. Distinct colicin M-like bacteriocin-immunity pairs in Burkholderia. Sci Rep. 2015; 5: 17368. https://doi.org/10.1038/srep17368 PMID: 26610609

19. Lupo A, Haenni M, Madec J-Y. Antimicrobial Resistance in Acinetobacter spp. and Pseudomonas spp. Antimicrobial Resistance in Bacteria from Livestock and Companion Animals. American Society of Microbiology; 2018. pp. 377–393. https://doi.org/10.1128/microbiolspec.arba-0007-2017 PMID: 30101740

20. Ellis CN, Cooper VS. Experimental adaptation of burkholderia cenocepacia to onion medium reduces host range. Appl Environ Microbiol. 2010; 76: 2387–2396. https://doi.org/10.1128/AEM.01930-09 PMID: 20154121

21. Parke JL, Gurian-Sherman D. Diversity of the burkholderia cepacia complex and implications for risk assessment of biological control strains. Annual Review of Phytopathology. Annu Rev Phytopathol; 2001. pp. 225–258. https://doi.org/10.1146/annurev.phyto.39.1.225 PMID: 11701865

22. Coombs JT, Franco CMM. Isolation and identification of actinobacteria from surface-sterilized wheat roots. Appl Environ Microbiol. 2003; 69: 5603–5608. https://doi.org/10.1128/AEM.69.9.5603-5608.2003 PMID: 12957950

23. Najnin RA, Shafrin F, Polash AH, Zaman A, Hossain A, Taha T, et al. A diverse community of jute (Corchorus spp.) endophytes reveals mutualistic host–microbe interactions. Ann Microbiol. 2015; 65: 1615–1626. https://doi.org/10.s13213-014-1001-1

24. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al. SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol. 2012; 19: 455–477. https://doi.org/10.1089/cmb.2012.0021 PMID: 22506599

25. Assela S, Keane TM, Otto TD, Newbold C, Berrian M. ABACAS: algorithm-based automatic contiguity of assembled sequences. Bioinformatics. 2009; 25: 1968–1969. https://doi.org/10.1093/bioinformatics/btp347 PMID: 19497936

26. Grant JR, Stothard P. The CGView Server: a comparative genomics tool for circular genomes. Nucleic Acids Res. 2008; 36. https://doi.org/10.1093/nar/gkn179 PMID: 18411202

27. Aziz RK, Bartels D, Best A, DeJongh M, Disz T, Edwards RA, et al. The RAST Server: Rapid annotations using subsystems technology. BMC Genomics. 2008; 9: 75. https://doi.org/10.1186/1471-2164-9-75 PMID: 18261238

28. Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, et al. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). Nucleic Acids Res. 2014; 42: D206. https://doi.org/10.1093/nar/gkt1226 PMID: 24293654

29. Martinez-Garcia PM, Lopez-Solanilla E, Ramos C, Rodriguez-Palenzuela P. Prediction of bacterial associations with plants using a supervised machine-learning approach. Environ Microbiol. 2016; 18: 4847–4861. https://doi.org/10.1111/1462-2920.13389 PMID: 27234490

30. Bin K, Shaw S, Steinke K, Villebro R, Ziemert N, Lee SY, et al. AntiSMASH 5.0: Updates to the secondary metabolite genome mining pipeline. Nucleic Acids Res. 2019; 47: W81–W87. https://doi.org/10.1093/nar/gkz310 PMID: 31032519

31. Chen L, Yang J, Yu J, Yao Z, Sun L, Shen Y, et al. VFDB: A reference database for bacterial virulence factors. Nucleic Acids Res. 2005; 33: D325–D328. https://doi.org/10.1093/nar/gk008 PMID: 15608208

32. Islam S, Akanda AM, Prova A, Islam MT, Hossain MM. Isolation and Identification of Plant Growth Promoting Rhizobacteria from Cucumber Rhizosphere and Their Effect on Plant Growth Promotion and Disease Suppression. Front Microbiol. 2016; 6: 1360. https://doi.org/10.3389/fmicb.2015.01360 PMID: 26869996

33. Yuan J, Raza W, Shen Q, Huang Q. Antifungal activity of Bacillus amyloliquefaciens NJN-6 volatile compounds against Fusarium oxysporum f. sp. cubense. Appl Environ Microbiol. 2012; 78: 5942–5944. https://doi.org/10.1128/AEM.01357-12 PMID: 22685147

34. Balouiri M, Sadiki M, Ibsnouda SK. Methods for in vitro evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis. Xi’an Jiaotong University; 2016. pp. 71–79. https://doi.org/10.1016/j.jpha.2015.11.005 PMID: 29403965

35. Elshafie H, Camele I, Racipori R, Scrano L, Iacobellis N, Bufo S. In Vitro Antifungal Activity of Burkholderia gladioli pv. agnicicola against Some Phytopathogenic Fungi. Int J Mol Sci. 2012; 13: 16291–16302. https://doi.org/10.3390/ijms131216291 PMID: 23208371

36. Navarro-González I, González-Barrio R, García-Valverde V, Bautista-Ortín A, Periago M. Nutritional Composition and Antioxidant Capacity in Edible Flowers: Characterisation of Phenolic Compounds by HPLC-DAD-ESI/MSn. Int J Mol Sci. 2014; 16: 805–822. https://doi.org/10.3390/ijms16010805 PMID: 25561232
37. Belozerskay TA, Gessler NN, Averyanov AA. Melanin Pigments of Fungi. Fungal Metabolites. Springer International Publishing; 2017. pp. 263–291. https://doi.org/10.1007/978-3-319-25001-4_29
38. Pearson WR. An introduction to sequence similarity ("homology") searching. Curr Protoc Bioinforma. 2013;Chapter 3: Unit3.1. https://doi.org/10.1002/0471250953.bi303142 PMID: 23749753
39. Parra-Cota Fl, Peña-Cabriales Jj, de los Santos-Villalobos S, Martínez-Gallardo NA, Delano-Frier JP. Burkholderia ambifaria and B. caribensis Promote Growth and Increase Yield in Grain Amananth (Amaranthus cruentus and A. hypochondriacus) by Improving Plant Nitrogen Uptake. Freitag NE, editor. PLoS One. 2014; 9: e88094. https://doi.org/10.1371/journal.pone.0088094 PMID: 24533068
40. Esmaeel Q, Jacquard C, Clément C, Sanchez L, Ait Barka E. Genome sequencing and traits analysis of Burkholderia strains reveal a promising biocontrol effect against grey mould disease in grapevine (Vitis vinifera L.). World J Microbiol Biotechnol. 2019; 35: 1–15. https://doi.org/10.1007/s11274-019-2613-1 PMID: 30792227
41. Liu C, Mou L, Yi J, Wang J, Liu A, Yu J. The Enoz Gene of Burkholderia cenocepacia Strain 71–2 is Involved in Phosphate Solubilization. Curr Microbiol. 2019; 76: 495–502. https://doi.org/10.1007/s00284-019-01642-7 PMID: 30798378
42. Lu SE, Novak J, Austin FW, Gu G, Ellis D, Kirk M, et al. Occidiofungin, a unique antifungal glycopeptide produced by a strain of Burkholderia contaminans. Biochemistry. 2009; 48: 8312–8321. https://doi.org/10.1021/bi900814c PMID: 19673482
43. Jung BK, Hong SJ, Park GS, Kim MC, Shin JH. Isolation of Burkholderia cepacia JFK9 with plant growth-promoting activity while producing pyrrolnitrin antagonistic to plant fungal diseases. Appl Bio Chem. 2018; 61: 173–180. https://doi.org/10.1007/s13765-018-0345-9
44. Land EJ, Ramsden CA, Riley PA. The mechanism of suicide-inactivation of tyrosinase: A substrate structure investigation. Tohoku J Exp Med. 2007; 212: 341–348. https://doi.org/10.1620/tjem.212.341 PMID: 17660699
45. Kumari N, Menghani E, Mithal R. GC-MS analysis of compounds extracted from actinomycetes AIA6 isolates and study of its antimicrobial efficacy. Indian J Chem Technol. 2019; 26: 362–370. Available: http://nopr.niscar.res.in/handle/123456789/49682
46. Chaukun X, Minghe M, Leming Z, Keqin Z. Soil volatile fungistasis and volatile fungistatic compounds. Soil Biol Biochem. 2004; 36: 1997–2004. https://doi.org/10.1016/j.soilbio.2004.07.020
47. Fujisawa S, Ishihara M, Murakami Y, Atsumi T, Kodama Y, Yokoe I. Predicting the biological activities of 2-methoxyphenol antioxidants: effects of dimers. In Vivo. 2007; 21(2):181–8. PMID: 17436566.
48. Mar A, Pripdeev P. Chemical composition and antibacterial activity of essential oil and extracts of Catharexylum spinosum flowers from Thailand. Nat Prod Commun. 2014; 9: 707–710. https://doi.org/10.1177/1934578x1400900532 PMID: 25026728
49. Meenakshi VK, Gomathy S, Senthamarai S. Paripoornaselvi M. Chamundeswari KP. GC-MS Determination of the Bioactive Components of Microcosmus Exasperatus heller, 1878. J Curr Chem Pharm Sci. 2012; 2: 271–276.
50. Teoh YP, Don MM, Ujang S. Media selection for mycelia growth, antifungal activity against wood-degrading fungi, and GC-MS study by Pycnoporus sanguineus. BioResources. 2011; 6: 2719–2731. https://doi.org/10.15376/biores.6.3.2719–2731
51. Hiramoto K, Nasuhara A, Michikoshi K, Kato T, Kikugawa K. DNA strand-breaking activity and mutagenicity of 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP), a Maillard reaction product of glucose and glycine. Mutat Res—Genet Toxicol Environ Mutagen. 1997; 395: 47–56. https://doi.org/10.1016/S1383-5718(97)00141-1
52. Al-Huqail AA, Behiry SI, Salem MZM, Ali HM, Siddiqui MH, Salem AZM. Antifungal, antibacterial, and antioxidant activities of Acacia saligna (Labill.) H. L. Wendl. Flower extract: HPLC analysis of phenolic compounds. Molecules. 2019;24: 707–710. https://doi.org/10.3390/molecules24040700 PMID: 30781352
53. Chang T-S. An Updated Review of Tyrosinase Inhibitors. Int J Mol Sci. 2009; 10: 2440–2475. https://doi.org/10.3390/ijms10062440 PMID: 19582213
54. Sladic D, Gasic M. Reactivity and Biological Activity of the Marine Sesquiterpene Hydroquinone Avalor and Related Compounds from Sponges of the Order Dictyoceratida. Molecules. 2006; 11: 1–33. https://doi.org/10.3390/molecules11010001 PMID: 17962742
55. Beulke S, Malkomes HP. Effects of the herbicides metazachlor and dinoterb on the soil microflora and the degradation and sorption of metazachlor under different environmental conditions. Biol Fertil Soils. 2001; 33: 467–471. https://doi.org/10.1007/s003740100354
56. Upadhyaya RS, Vandavasi JK, Kardile RA, Lahore S V., Dixit SS, Deokar HS, et al. Novel quinoline and naphthalene derivatives as potent antimycobacterial agents. Eur J Med Chem. 2010; 45: 1854–1867. https://doi.org/10.1016/j.ejmech.2010.01.024 PMID: 20137835
57. Hakozaki T, Laughlin T, Zhao S, Wang J, Deng D, Jewell-Motz E, et al. A regulator of ubiquitin-proteasome activity, 2-hexyldecanol, suppresses melanin synthesis and the appearance of facial hyperpigmented spots. Br J Dermatol. 2013; 169: 39–44. https://doi.org/10.1111/bjd.12364 PMID: 23786619

58. Hadi I, Mashkoor Hussein H. Antimicrobial activity and spectral chemical analysis of methanolic leaves extract of adiantum capillus-veneris using GC-MS and FT-IR spectroscopy. Int J Pharmacogn Phytochem Res. 2016; 8(3): 369–385. Available: www.ijprr.com

59. Vivekraj AV P., Anand gideon. and V. Analysis of Phytochemical constituents of the chloroform extracts of Abutilon hirtum (Lam.) Sweet using GC-MS Method. Int J Pharmacol Res. 2019; 09: 167–171. https://doi.org/10.7439/ijpr

60. Ser HL, Palanisamy UD, Yin WF, Abd Malek SN, Chan KG, Goh BH, et al. Presence of antioxidative agent, Pyrrolo[1,2-\textasteriskcentered]pyrazine-1,4-dione, hexahydr- in newly isolated Streptomyces mangrovisoli sp. nov. Front Microbiol. 2015; 6. https://doi.org/10.3389/fmicb.2015.00854 PMID: 26347733

61. Kannabiran DK. Bioactivity of Pyrrolo[1,2-\textasteriskcentered]pyrazine-1,4-dione, hexahydr-0-(phenylmethyl)- Extracted from Streptomyces sp. VITPK9 Isolated from the Salt Spring Habitat of Manipur, India. Asian J Pharm Free full text Artic from Asian J Pharm. 2016; 10: 265. https://doi.org/10.22377/AJP.V10I0.865

62. Kiran GS, Priyadharsini S, Sajayan A, Ravindran A, Selvin J. An antibiotic agent pyrrolo[1,2-\textasteriskcentered]: A] pyra- zine-1,4-dione,hexahydrisolated from a marine bacteria Bacillus tequilensis MS145 effectively controls multi-drug resistant Staphylococcus aureus. RSC Adv. 2018; 8: 17837–17846. https://doi.org/10.1039/c8ra00820e

63. Pérez-Picaso L, Olivo HF, Argo-tte-Ramos R, Rodrı́guez-Gutiérrez M, Rios MY. Linear and cyclic dipeptides with antimarial activity. Bioorganic Med Chem Lett. 2012; 22: 7048–7051. https://doi.org/10.1016/bmcl.2012.09.094 PMID: 23084276

64. Nagella P., Chemical constituents, larvicidal effects and antioxidative activity of petroleum ether extract from seeds of Coriandrum sativum L. J Med Plants Res. 2012; 6. https://doi.org/10.5897/jmpr11.992

65. Chandrasekaran M, Senthilkumar A, Venkatesalu V. Antibacterial and antifungal efficacy of fatty acid methyl esters from the leaves of Sesuvium portulacastrum L. Eur Rev Med Pharmacol Sci. 2011; 15(7): 775–780. PMID: 21780546.

66. Mahamuni SV. Antifungal Trait of Burkholderia gladioli Strain VIMP02 (JQ811557). Int J Sci Res. 2015; 4: 2059–2064. Available: https://www.ijr.net/search_index_results_paper.php?id=SUB157864

67. Samling B, John Umaru I. Phytochemical screening, antioxidiant, antifungal potentials of Acacia auriculiformis florescent composition. J Anal Pharm Res. 2018; 7. https://doi.org/10.15406/japlr.2018.07.00296

68. Aftab A, Yousaf Z, Javaid Z, Riaz N, Younas M, et al. Antifungal activity of vegetative methanoic extracts of Nigella sativa against Fusarium oxysporum and Macrophomina phaseolina and its phytochemical profiling by GC-MS analysis. Int J Agric Biol. 2019; 21: 569–576. https://doi.org/10.17957/ijab/15.0930

69. Torres MJ, Brandon CP, Petroselli G, Erra-Ballels R, Audisio MC. Antagonistic effects of Bacillus subtilis subsp. subtilis and B. amyloliquefaciens against Macrophomina phaseolina: SEM study of fungal changes and UV-MALDI-TOF MS analysis of their bioactive compounds. Microbiol Res. 2016; 182: 31–39. https://doi.org/10.1016/j.micres.2015.09.005 PMID: 26686611

70. Berendsen RL, Pieterse CMJ. Bakker PAHM. The rhizosphere microbiome and plant health. Trends in Plant Science. Trends Plant Sci; 2012. pp. 478–486. https://doi.org/10.1016/j.tplants.2012.04.001 PMID: 22564542

71. Shannugam V, Kanoujia N, Singh M, Singh S, Prasad R. Biocontrol of vascular wilt and corn rot of gladiolus caused by Fusarium oxysporum f. sp. gladioli using plant growth promoting rhizobacterial mixture. Crop Prot. 2011; 30: 807–813. https://doi.org/10.1016/j.cropro.2011.02.033

72. Pineda A, Dicke M, Pieterse CMJ, Pozo MJ. Beneficial microbes in a changing environment: are they always helping plants to deal with insects? Biere A, editor. Funct Ecol. 2013; 27: 574–586. https://doi.org/10.1111/1365-2435.12050

73. Hayat R, Ali S, Amara U, Khalid R, Ahmed I. Soil beneficial bacteria and their role in plant growth promotion: A review. Annals of Microbiology. BioMed Central; 2010. pp. 579–598. https://doi.org/10.1007/s13213-010-0117-1

74. Holden MTG, Seth-Smith HMB, Crossman LC, Sebaihia M, Bentley SD, Cerdeño-Tárraga AM, et al. The genome of Burkholderia cenocepacia J2315, an epidemic pathogen of cystic fibrosis patients. J Bacteriol. 2009; 91: 261–277. https://doi.org/10.1128/JB.01230-08 PMID: 19391103

75. Lim JY, Lee TH, Baek HY, Yang DC, Kim M, Hwang I. Complete genome sequence of Burkholderia glumae BGR1. J Bacteriol. 2009; 191: 3758–3759. https://doi.org/10.1128/JB.00349-09 PMID: 19329631
76. Nunvar J, Kalfertova L, Bloodworth RAM, Kolar M, Degrossi J, Lubovich S, et al. Understanding the Pathogenicity of Burkholderia contaminans, an Emerging Pathogen in Cystic Fibrosis. Bevivino A, editor. PLoS One. 2016; 11: e0160975. https://doi.org/10.1371/journal.pone.0160975 PMID: 27512997

77. Urban TA, Goldberg JB, Forstner JF, Sajjan US. Cable pili and the 22-kilodalton adhesin are required for Burkholderia cenocepacia binding to and transmigration across the squamous epithelium. Infect Immun. 2005; 73: 5426–5437. https://doi.org/10.1128/IAI.73.9.5426-5437.2005 PMID: 16113259

78. Angus AA, Agapakis CM, Fong S, Yerrapragada S, Estrada-de los Santos P, Yang P, et al. Plant-Associated Symbiotic Burkholderia Species Lack Hallmark Strategies Required in Mammalian Pathogenesis. van Schaik W, editor. PLoS One. 2014; 9: e83779. https://doi.org/10.1371/journal.pone.0083779 PMID: 24416172

79. Schwarz S, Singh P, Robertson JD, LeRoux M, Skerrett SJ, Goodlett DR, et al. VgrG-5 is a Burkholderia type VI secretion system-exported protein required for multinucleated giant cell formation and virulence. Infect Immun. 2014; 82: 1445–1452. https://doi.org/10.1128/IAI.01368-13 PMID: 24452686

80. Deng P, Wang X, Baird SM, Showmaker KC, Smith L, Peterson DG, et al. Comparative genome-wide analysis reveals that Burkholderia contaminans MS14 possesses multiple antimicrobial biosynthesis genes but not major genetic loci required for pathogenesis. Microbiologyopen. 2016; 5: 353–369. https://doi.org/10.1002/mbo3.333 PMID: 26769582

81. Corbett CR, Burtnick MN, Kooi C, Woods DE, Sokol PA. An extracellular zinc metalloprotease gene of Burkholderia cepacia. Microbiology. Society for General Microbiology; 2003. pp. 2263–2271. https://doi.org/10.1099/mic.0.26243-0 PMID: 12904566

82. Xu X-H, Su Z-Z, Wang C, Kubicek CP, Feng X-X, Mao L-J, et al. The rice endophyte Harpophora oryzae genome reveals evolution from a pathogen to a mutualistic endophyte. Sci Rep. 2014; 4: 5783. https://doi.org/10.1038/srep05783 PMID: 25048173

83. Corbett CR, Burtnick MN, Kooi C, Woods DE, Sokol PA. An extracellular zinc metalloprotease gene of Burkholderia cepacia. Microbiology. Society for General Microbiology; 2003. pp. 2263–2271. https://doi.org/10.1099/mic.0.26243-0 PMID: 12904566

84. Quan CS, Zheng W, Liu Q, Ohta Y, Fan SD. Isolation and characterization of a novel Burkholderia cepacia with strong antifungal activity against Rhizoctonia solani. Appl Microbiol Biotechnol. 2006; 72: 1276–1284. https://doi.org/10.1007/s00253-006-0425-3 PMID: 16708194

85. Wheatley RE. The consequences of volatile organic compound mediated bacterial and fungal interactions. Antonie van Leeuwenhoek, Int J Gen Mol Microbiol. 2012; 81: 357–364. https://doi.org/10.1023/a:1020592802234 PMID: 12448734

86. Schalchi H, Hormazabal B, Becerra J, Birkett M, Alvear M, Vidal J, et al. Antifungal activity of volatile metabolites emitted by mycelial cultures of saprophytic fungi. Chem Ecol. 2011; 27: 503–513. https://doi.org/10.1080/02757540.2011.596832

87. Gao Z, Zhang B, Liu H, Han J, Zhang Y. Identification of endophytic Bacillus velezensis ZSY-1 strain and antifungal activity of its volatile compounds against Alternaria solani and Botrytis cinerea. Biocontrol. 2017; 105: 27–38. https://doi.org/10.1016/j.biocon.2016.11.007

88. Kumari N, Menghani E, Mithal R. Bioactive Compounds characterization and Antibacterial Potentials of Actinomycetes isolated from Rhizospheric soil. J Sci Ind Res. 2019; 78: 793–798. Available: http://nopr.niscair.res.in/handle/123456789/51187

89. 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. https://doi.org/10.1046/j.1365-2672.1998.00473.x PMID: 9721657

90. Zhang P, Zhou S, Wang G, An Z, Liu X, Li K, et al. Two transcription factors cooperatively regulate DHN melanin biosynthesis and development in Pestalotiopsis fici. Mol Microbiol. 2019; 112: 649–666. https://doi.org/10.1111/mmi.14281 PMID: 31116900

91. VIDAL-CROS A, VIVIANI F, LABESSE G, BOCCARA M, GAUDRY M. Polyhdroxynaphthalene reductase involved in melanin biosynthesis in Magnaporthe oryzae Purification, cDNA cloning and sequencing. Eur J Biochem. 1994; 219: 985–992. https://doi.org/10.1111/j.1432-1033.1994.tb18581.x PMID: 8112949

92. Eisenman HC, Casadevall A. Synthesis and assembly of fungal melanin. Applied Microbiology and Biotechnology. NIH Public Access; 2012. pp. 931–940. https://doi.org/10.1007/s00253-011-3777-2 PMID: 22173481

93. Almeida-Paes R, Frases S, Fialho Monteiro PC, Gutierrez-Galhardo MC, Zancopé-Oliveira RM, Nosanchuk JD. Growth conditions influence melanization of Brazilian clinical Sporothrix schenckii isolates. Microbes Infect. 2009; 11: 554–562. https://doi.org/10.1016/j.micinf.2009.03.002 PMID: 19328867