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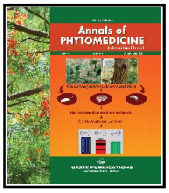

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Alcaligenes faecalis strain SASBG203: A novel biocontrol agent against *Colletotrichum orbiculare*

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Abstract

Bottle gourd is one of the important cucurbitaceous vegetables widely consumed in Indian households. Many fungal diseases affect its cultivation and one among them is stem splitting caused by *Colletotrichum orbiculare*. This pathogen causes a range of symptoms, *viz*., leaf spots, vertical water-soaked streaks on petioles, stems, crotches and splitting of stem at crown region. Farmers resort to indiscriminate use of fungicides for controlling the disease which poses the threat of chemical residues in the produce.The aim of this study is to come out with ecofriendly approach like use of native bacterial endophyte from bottle gourd to manage the disease. Of the endophytes isolated, *Alcaligenes faecalis* strain SASBG203 performed well against the pathogen. The mycelial growth of *Colletotrichum orbiculare* was inhibited to the tune of 71.08 per cent in dual culture experiments compared to the mycelial growth in control plates. The profiling of soluble metabolites was performed through gas chromatography and mass spectrometry (GC-MS) in control plates with fungus alone, the plates showing inhibition zone and from empty PDA plates. Twelve metabolites, *viz*., Shikimic acid; 2-Propenoic acid, 3-[5-(2-hydroxypropyl)-2,2-dimethyl-1,3-dioxolan-4-yl]-, TBDMS derivative; dl-Mevalonic acid lactone; 4H-Pyran-4-one, 5-hydroxy-2-(hydroxymethyl)-; 2,4-Hexadienoic acid, ethyl ester; Glycerin; Catechol; cis-Vaccenic acid; 9,12-Octadecadienoic acid (Z, Z)-; Ethanol, 2-[(2-aminoethyl) amino] and trans-1,10-Dimethyl-trans-9-decalinol were actively expressed in the interaction zone. The biosynthetic pathway topology and pathway impact assessment revealed glycerolipid metabolism and phenylalanine, tyrosine and tryptophan biosynthesis with impact values of 0.29 and 0.08 were unique in the interaction of *Alcaligenes faecalis* strain SASBG203 with *C. orbiculare*. The over representation analysis of soluble biomolecules from interaction zone against human metabolome suggested they were highly metabolized in bile acid biosynthesis, steroid biosynthesis and fatty acid metabolism. These compounds in interaction zone can be seen as potential antifungal agents against *C. orbiculare in* further studies.

1. Introduction

Bottle gourd (*Lagenaria siceraria* (Molina) Standl.) is a cucurbitaceous vegetable and it is one of the chief culinary vegetables in many tropical and temperate regions around the world. The edible portion, fruit contains phytochemicals like vitamins, proteins, choline, minerals, terpenoids, flavonoids, *etc*., Several bioactive compounds have been isolated from *L. siceraria*, including triterpenoids, sterols, cucurbitacins, flavones, C-glycosides and β-glycosides. Researchers have evaluated use of various parts of the plant as antianxiety, antidepressant, diuretic, antimicrobial, cytotoxic, antihyperlipidemic, cardio protective, analgesic, anti-inflammatory, antihelminthic, antihyperglycaemic, antihepatotoxic, antiurolithiasis, antistress, antiulcer, anticancer, hepatoprotective, anthelmintic, immunomodulatory, and antioxidant (Zahoor *et al*., 2021). Despite its economic and medicinal importance, the crop is susceptible to a variety of fungal, bacterial, and viral diseases, including downy mildew, powdery mildew, leaf spots, cucumber mosaic virus (CMV), *etc*. (Zitter *et al*., 1998; Saha, 2002).

In the last few decades, microbial communities have been deployed in various fields of agriculture and horticulture as biocontrol agents, biofertilizers, bioinoculants and as stress modulators. Endophytes are microorganisms residing inside the host tissues without causing any apparent signs of infection. With the advanced omics and technologies, very few endophytic organisms have been cultured and assessed for their efficiency in suppressing plant diseases. Endophytic communities have immense potential as biocontrol agents and intern encourage plant growth and thereby seen as potent option for non-toxic management of plant diseases. Endophytes

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are preferred over other plant growth promoting rhizobacteria for their better survival and adaptation against biotic and abiotic stresses.

Endophytic bacterial community from twigs of bottle gourd was isolated and evaluated against bottle gourd stem splitting organism *C. orbiculare* under lab conditions and non-volatile metabolites expressed in inhibition zone were determined using GC-MS analysis. Further, efforts were made to ascertain the involvement of such metabolites in various fungal metabolic pathways and in more than one metabolic pathway of fungus.

2. Materials and Methods

2.1 Pathogen isolation

Symptomatic samples collected from affected fields were used for isolation of pathogen following the procedure of Nuraini and Latiffah (2018) with slight modifications. Small 5 mm bits from affected regions were cut with sterile surgical blade and surface sterilized in 1.0 per cent sodium hypochlorite solution for three minutes and then thoroughly rinsed in three changes of sterilized distilled water. The surface sterilized specimens were dried on pre sterilized blotting paper for five minutes. These air-dried bits were placed on potato dextrose agar (PDA) medium plates and incubated at ambient temperature. Single hyphal tip of the fungus was transferred to freshly prepared PDA plates for purification.

2.2 Proving the molecular identity of the pathogen

The fungal genomic DNA was extracted using CTAB method according to Mishra *et al.* (2014). The fungus was characterized using nuclear ribosomal internal transcribed spacer (nrITS) primers, *viz.*, ITS1 (5' CTTGGTCATTTAGAGGAAGTAA 3') and ITS4 (5' TCCTCCGCT TATTGATATGC 3') (White *et al.*, 1990). The reaction was carried out in Eppendorf ® mastercycler with a 40 µl reaction mixture consisting of 20 µl Taq DNA polymerase master mix Red (Ampliqon®), 4 µl forward primer, 4 µl reverse primer, 4 µl double distilled water and 8 µl of genomic DNA. The PCR conditions include an initial denaturation at 95°C for 4 min, followed by 35 cycles of denaturation at 95^{0}C for 4 min; primer annealing at 62^{0}C for 1 min; extension at 72^{0}C for 2 min and final extension at 72^{0}C for 8 min. 5 µl of amplified PCR product was loaded on to 1.2% (w/v) ethidium bromide-stained agarose gel and electrophoresed at 75 V for 1.5 h. The gel was visualized under UV light (UVITECH, Cambridge Inc.). Remaining PCR product was sequenced in ABI-3730 Prison automated DNA analyser EBT Ver. 3.1 (Barcode Bio Science, Bangalore, India). The sequencing result was compared with NCBI-GenBank database using BLASTN algorithm (www.ncbi.nlm.nih.gov) and the same was submitted to obtain accession number.

2.3 Endophyte extraction

Endophytes were extracted from the young tender bottle gourd plant parts, following the protocol of Sriskandarajah *et al.* (1993) with slight modifications. Small bits of bottle gourd leaves were aseptically cut and surface sterilized in 1.0 per cent sodium hypochlorite for 3 min and washed in three changes of sterile distilled water. Then the bits were transferred in 70 per cent ethyl alcohol for one minute and again washed in three changes of sterile distilled water. Surface sterilized plant bits were blotted dry in sterile blotting paper. 1.0 gm of surface sterilized bits was crushed with 5 ml of peptone salt buffer in a sterilized pestle and mortar. 1 ml of suspension was pour plated in nutrient agar medium and incubated at 35^{0}C for development of bacterial colonies. Distinct bacterial colonies were isolated and streaked in pure form on nutrient agar plate. For sterility check, water collected from the final wash process was plated on nutrient agar plates and cultivated at 35°C for 24 h (Zhan-Bin *et al.*, 2013).

2.4 Dual culture assay

The pathogen used in this study was *Colletotrichum orbiculare* maintained at 25^{0}C after subculturing every 10 days on PDA (Potato Dextrose Agar) plates. The procedure outlined by Irma (2018) was followed with slight modifications. A 5 mm fungal pathogen disc was inoculated in the center of PDA plates and 5µl of 8 h old actively growing bacterial culture was inoculated on four sides of fungal disc 5 mm away from the rim of petriplate. PDA plates with only fungal disc were maintained as control plates. The diameter of fungal discs in dual culture plates were measured once the fungal disc growth in control plates was fully occupied. Per cent inhibition was calculated using the following formula:

$$\text{PDI} = \frac{\text{Growth in control} - \text{Growth in treatment}}{\text{Growth in control}} \times 100$$

The treatments were replicated thrice and the PDI was analyzed and compared the means in OPSTAT software with CRD experiment.

2.5 Genomic DNA extraction from bacterial endophyte

The total bacterial DNA was extracted by CTAB method, following the procedure of Doyle and Doyle (1990). The bacteria was inoculated in 100 ml of nutrient broth and incubated for 24 h at 37^{0}C in shaker at 180 rpm. One ml of broth was taken into 1.5 ml Eppendorf tube and centrifuged at 7000 rpm for 10 min to retain pellet. One ml of broth was added to same Eppendorf tube and centrifuged at 7000 rpm for 10 min to retain pellet. One ml of sterile water was added to pellet and centrifuged at 7000 rpm for10 min to remove traces of broth and the pellet was suspended in 675 µl of genomic DNA buffer (CTAB) and incubated at 37^{0}C for 30 min with vortexing at every 10 min interval. 75 µl of 10 per cent SDS was added and incubated at 65^{0}C for 2 h with vortexing at every 10 min. The tube was centrifuged at 11000 rpm for 10 min at 4^{0}C and the supernatant was collected in fresh Eppendorf tube to which equal volume of phenol: chlorophorm: isoamylalcohol (25:24:1) was added. The tube was inverted many times and centrifuged at 11000 rpm for 10 min at 4^{0}C that led to formation of three phases. Aqueous phase was transferred to new Eppendorf tube and the DNA was precipitated by adding 0.6 volume of Isopropanol with incubation for one hour at –20^{0}C. The tube was centrifuged at 12000 rpm for 15 min at 4^{0}C to retain DNA pellet which was washed in 70 per cent ethyl alcohol twice. The DNA pellet was air dried and suspended in double sterilized distilled water for further use.

2.6 16S rRNA gene amplification by PCR and phylogenetic analysis

The bacterial endophyte aggressive against test fungus in dual culture assay was identified by PCR amplification with universal 16S rRNA primers 27 F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') with following PCR conditions:

initial denaturation at 95^{0}C for 5 min, denaturation at 94^{0}C for 30 seconds, primer annealing at 58^{0}C for 30 seconds, extension at 72^{0}C for 40 seconds and final chain extension at 72^{0}C for 10 min (Watanabe *et al.* 2001). The PCR was run with a 40 µl reaction mixture containing 20 µl Amplicon® oligonucleotide mixture, 4 µl forward primer, 4 µl reverse primer, 4 µl double sterilized distilled water and 8 µl of bacterial DNA of 54 ng/µl concentration. 5 µl of amplified PCR product was loaded on to 1.2% (w/v) ethidiumbromide stained agarose gel and electrophoresed at 75 V for 1.5 h. The gel was visualized under UV light (UVITECH, Cambridge Inc.) for amplification of said region between the primers.

2.7 Sequencing and analysis of 16S rRNA gene sequences of endophytic bacteria

Amplified PCR product was sequenced in ABI-3730 Prison automated DNA analyser EBT Ver. 3.1 (Barcode Bio Science, Bangalore, India). The resulting sequence was edited with BIOEDIT software to obtain full length sequence of 16S rRNA of the endophyte. The sequence was compared with NCBI-GenBank database using blastn algorithm (www.ncbi.nlm.nih.gov) and was submitted to obtain accession number. The nucleotide sequence was searched for sequence homology using BLAST search against Gen Bank database (http://www.ncbi.nih.gov/BLAST). The related endophyte bacterial 16S rRNA gene sequences retrieved from the GenBank database were used for phylogenetic analysis.The phylogenetic tree was constructed with bootstrap for 1000 times using the neighbor-joining method (Saitou and Nei, 1987; Tamura *et al.*, 2011) and compared in MEGA XI software.

2.8 Secondary metabolite extraction and GC-MS analysis

Secondary metabolites responsible for actual inhibition of pathogen fungus were estimated in GC-MS analysis. For obtaining samples from inhibition zones procedure of Cawoy *et al.* (2014) was followed with slight modifications. 10 g of agar samples were taken from the inhibition zone and mixed with 100 ml of acetonitrile: water (1:1; v/v). This mixture was sonicated (Bandelin Sonoplus HD 2070) twice during 30 s at 30% of the power of the device. The samples were homogenized (vortex) then, centrifuged and filtered to eliminate any agar particles. Obtained filtrates were analysed using GC-MS in the instrument Claurus SQ8C of Perkin Elmer, USA. This instrument had DB-5 MS capillary standard non-polar column with dimensions: 30 mts length x ID 0.25 mm x film 0.25 mm IM. Helium was used as carrier gas with injection rate at 1 micro liter/min. The obtained peaks from PDA where fungus alone was grown, interaction zone and from PDA where endophyte alone was grown were compared and metabolites specifically expressed in successful endophyte fungal interaction zone were computed. NIST ms/ms database, mainlib and replib EI libraries were the inbuilt libraries found in the data system for searching and matching the spectrum. Those compounds with spectral fit values equal to or greater than 700 were considered for identification based on the MS data library and comparing the spectrum obtained through.

2.9 Plant growth promotion parameters of endophyte (qualitative tests only)

Production of IAA, catalase, amylase, cellulase, nitrogen fixation and phosphate solubilization were carried out according procedures laid down in Digar Singh *et al.* (2013). For assaying IAA production, endophytic bacteria was inoculated in YMD broth tube aseptically and incubated at 35^{0}C. 1 ml of supernatant was mixed with 2 ml of Salkowski's reagent (2% 0.5 $FeCl_3$ in 35% $HClO_4$ solution) and maintained in dark. The optical density (OD) was recorded at 530 nm after 30 min and 120 min to determine IAA production. For assaying catalase production, endophytic bacteria was streaked on nutrient agar slant and incubated at 35^{0}C for 24 h. 1.0% hydrogen peroxide was flooded on the actively growing bacteria to observe bubble formation. For assaying amylase production, endophytic bacteria was streaked on nutrient agar media enriched with starch@ 2 g/l and incubated at 35^{0}C for 24 h. Next day, the plates were flooded with iodine reagent to observe clear zone around streaks. For assaying cellulase production, endophytic bacteria was streaked on CMC agar media plates and incubated at 35^{0}C for 24 h. Production of clear zone around colonies and degradation of Cango red indicated a positive reaction. Similarly, production of clear zone around bacterial streaks on YEMA plates (yeast extract mannitol agar) and on Pikovaskya agar media plates after incubation at 35^{0}C for 24 h indicated bacterial production of nitrogen and phosphorous from the media, respectively.

Ammonia production was assayed according to Hansen (1930). Endophytic bacteria was inoculated in nutrient broth and incubated at 35^{0}C for 24 h. Development of yellow colour upon addition of Nessler's reagent indicated ammonia production. Production of acetoin and 2,3 butanediol were done according to procedures of Shanmugaraj *et al.* (2021). The endophytic bacteria was inoculated in sterilized MRVP broth tubes and incubated at 35^{0}C for 24 h. Development of cherry red colour after addition of VP-1 reagent (alpha naphthol) and VP-2 reagent (40% potassium hydroxide) reagent indicated positive reaction

Production of chitinase was assayed according to Chernin *et al.* (1995). The endophytic bacteria was streaked on colloidal chitin agar medium and incubated at 35^{0}C for 24 h. Production of clear zone around streaked colonies indicated a positive reaction. Zinc solubilization was assayed according to Fasim *et al.* (2002). Endophytic bacteria was streaked on Tris-minimal medium supplemented separately with zinc oxide (ZnO) [1.244 /l] = 15.23 mM and zinc phosphate $Zn_3(PO_4)_2$[1.9882 g/l] = 5.0 mM at a concentration equivalent to 0.1% Zn (Fasim *et al.*, 2002). After incubation at 35^{0}C for 24 h, production of clear zone around colonies indicated a positive reaction. Production of protease was assayed according to Denizci *et al.* (2004). Endophytic bacteria was streaked on skim milk agar (SMA: 100% sterile of 900 ml media tryptic soy agar (TSA), 10% concentration of 100 ml of sterile skim milk) plates and incubated at 35^{0}C for 24 h. Production of clear zone around colonies indicated a positive reaction

2.10 Statistical analysis

The design of the experiment and the statistical analysis was performed using OPSTAT software package. The comparative analysis of volatile compounds was followed using ClustVis online (https://www.biit.cs.ut.ee/clustvis/). The metabolic pathway enrichment analysis and metabolite over representation analysis was performed through Metabo Analyst 5.0 (http:// www.metabo analyst.ca/).

3. Results

3.1 Pathogen isolation and identification

The fungal growth from the infected twigs was subcultured on to fresh PDA plates. The growth of the fungus on PDA medium was whitish for seven days. Later, turned to olivaceous green and then brown colour. Dark brown to orange colour spore masses produced as ooze are seen after 30 days of growth. The conidia were hyaline, single celled, oval with rounded ends (Figure.1). The pathogen produced all the typical symptoms, *viz*., leaf spots, vertical water-soaked streaks on petioles, stems, crotches and splitting of stem at crown region. Subsequently, same pathogen had been reisolated fulfilling Koch's postulates.

The fungal DNA was clearly amplified with ITS1 and ITS4 primers, the amplified product was sequenced and compared in NCBI blastn search to establish the molecular identity of the pathogen as *C. orbiculare*. The sequence was submitted in NCBI database with accession number ON398802.

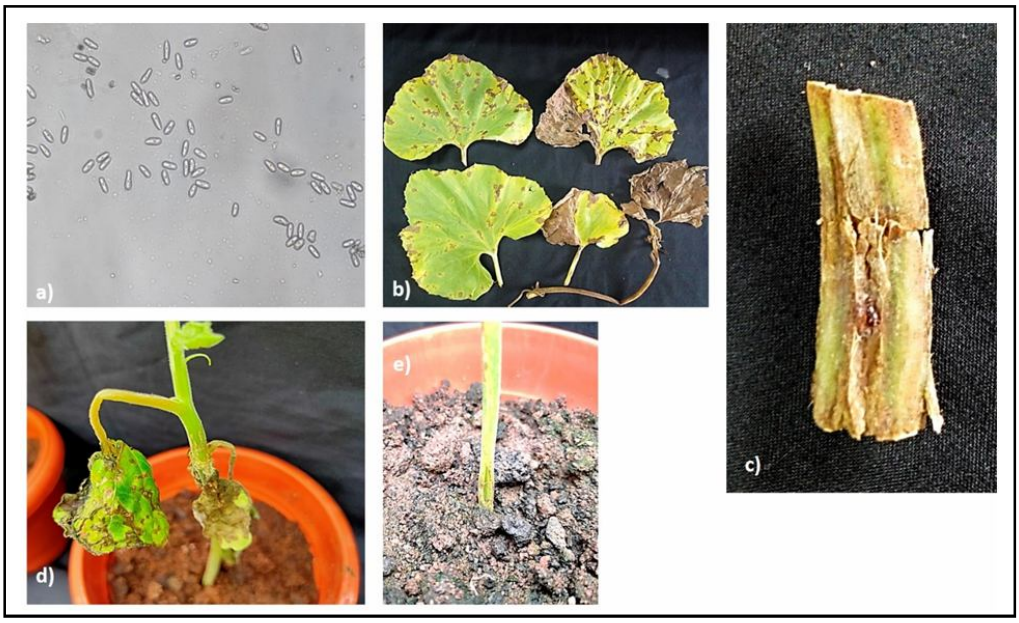

Figure 1: (a): Conidia of *C. orbiculare* (b): Leaf spots, (c): Stem splitting at field level, (d) Pathogenicity test showing leaf spots and (e): Stem splitting

Table 1: *In vitro* per cent inhibition of *C. orbiculare* mycelial growth by *Alcaligenes faecalis* SASBG203

Treatment number	Treatment (Bacterial endophytes)	Mean of highest diameters of fungal growth in petri plate (cm)			PDI
		R1	R2	R3	
1	SASBG 201	2.45	1.95	1.65	73.29
2	SASBG 203	2	2.7	1.85	71.08
3	SASBG 206	2.2	2.3	2.4	69.54
4	SASBG 208	7	6	4.5	22.74
5	SASBG 214	2.55	2.5	2.5	66.67
6	SASBG 215	2	1.75	2.25	73.51
7	SASBG 217	2	2.2	2.2	71.74
8	SASBG 221	3.35	3.2	3.35	56.29
9	SASBG 223	3	3.35	3.4	56.95
10	SASBG 202	5.1	5	5.35	31.79
11	SASBG 204	5.1	4.9	5.2	32.89
12	SASBG 205	4.4	4.9	4.35	39.74
13	SASBG 207	5.65	5.65	6	23.62
14	SASBG 209	6	6.1	6.1	19.65
15	SASBG 210	6.1	6.1	5.7	20.97
16	Control	7.65	7.5	7.5	
	C.D.	0.914			
	SE(m).	0.316			
	C.V.	4.800			

3.2 Dual culture test results and plant growth promotion assays

Fifteen bacterial endophytes were isolated and tested for efficacy againsts *C. orbiculare* in dual culture tests. The endophytes SASBG201, SASBG203 and SASBG215 recorded 73.29, 71.08 and 73.51 per cent reduction of radial mycelial growth over control, respectively (Table. 1 and Figure 2).

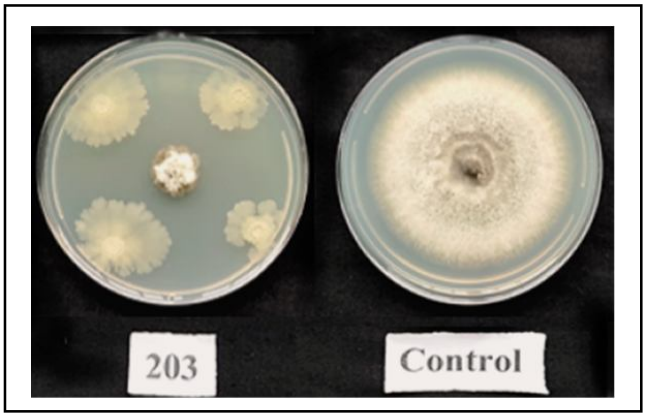

Figure 2: Mycelial growth inhibition of *C. orbiculare* by *Alcaligenes faecalis* strain SASBG203 in dual culture experiments.

The strain SASBG203 was further analyzed for plant growth promoting characteristics where, it showed positive for production of IAA, amylase, acetoin, nitrogen, phosphate, cellulase, protease, and for 2, 3, butane diol. The bacterial strain was negative for production of catalase, ammonia, chitinase and for Zn solubilization.

3.3 Identification of bacterial endophyte

The DNA from bacterial endophyte strain SASBG203 was amplified with 16S rRNA universal primers 27 F and 1492 R which showed a clear band at 1500 bp in agarose gel electrophoresis (Figure 3).

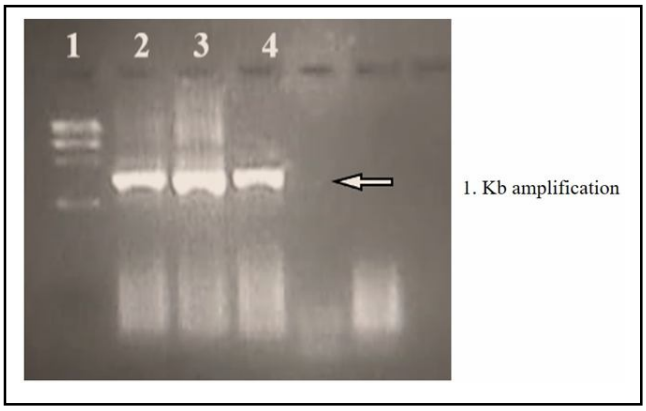

Figure 3: Amplification of Alcaligenes faecalis strain SASBG 203 bacterial DNA with universal primers 27F and 1492R : Well 1-1Kb ladder, 2-SASBG201, 3-SASB G203,4-SASBG215.

The BLASTn search of sequenced product NCBI database showed a close similarity with *Alcaligene faecalis*.The sequence was submitted in NCBI database with accession number OM980092 with strain name SASBG203. The phylogenetic tree built with 16 other *Alcaligenes* spp. sequences showed a close cluster formation in MEGA XI software (Figure. 4).

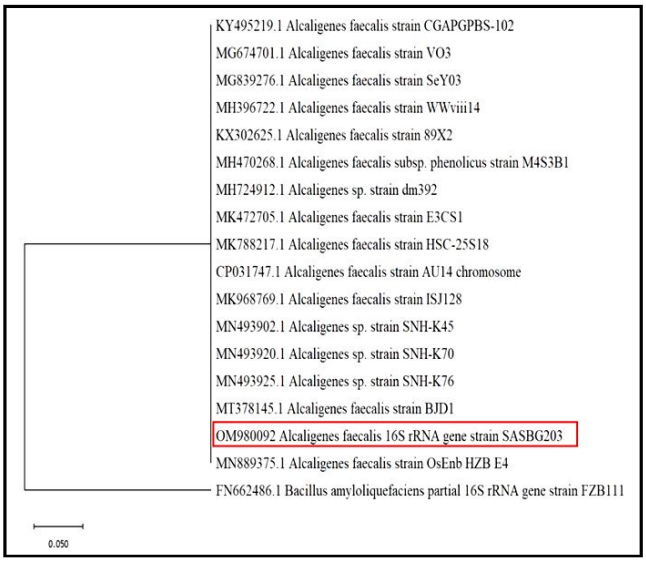

Figure 4: Neighbour joining phylogenetic tree of *Alcaligenes faecalis* strain SASBG203 based on 16s rRNA gene sequences retrieved from NCBI database

3.4 Profiling of bioactive antifungal metabolites by gas chromatography and mass spectrometry

The dual culture bioassay confirmed the antifungal activity of *Alacligenes feacalis* strain SASBG203 against *C. orbiculare* and it was certainly due to soluble non-volatile biomolecules produced by the endophytic bacterium. To further identify the chemical nature of the metabolites, gas chromatography and mass spectrometry (GC-MS) profiling was employed. The NIST library identified most of the metabolites by similarity index of 85% and most probable hits. A total of 1771 compounds were profiled from different treatment combinations, *viz*., *Alcaligenes faecalis* alone, *C. orbiculare* alone and their interaction in dual culture assay.

In *A. faecalis* alone, produced 67 hits with 967 compounds and the unique compounds profiled were N8-Acetylspermidine; phenol; cytidine 2',3'-cyclic monophosphoric acid; (S)-5-hydroxymethyl-2[5H]-furanone; α-D-glucopyranose, 1,6-anhydro-; 6-hydroxy-4-methyl-3-phenylcoumarin; 1-pentanol, 2-methyl-, acetate; 2-t-butyl-4-methyl-5-oxo-[1,3]dioxolane-4-carboxylic acid; sucrose; butanoic acid, 2-methyl-, ethyl ester; 6-oxa-bicyclo[3.1.0] hexan-3-ol; d-glycero-d-ido-heptose.

In *C. orbiculare* alone, produced 57 hits with 667 compounds and the unique compounds profiled were dihydro-2(3H)-thiophenone; 2-cyclopenten-1-one, 2-hydroxy-; val-Met-Lys; 5-oxotetra hydrofuran-2-carboxylic acid; 1,2,3-propanetriol, 1-acetate; lactose; erythritol; 2,4-hexadienedioic acid; DL-Arabinitol.

The interaction of *A. faecalis* and *C. orbiculare* in dual culture assay produced 35 hits with 142 compounds and the unique compounds profiled were shikimic acid; 2-propenoic acid; dl-mevalonic acid lactone; 4H-pyran-4-one, 5-hydroxy-2-(hydroxymethyl)-; 2,4-hexadienoic acid, ethyl ester; glycerin; catechol; cis-vaccenic acid; 9,12-octadecadienoic acid (Z,Z)-; ethanol, 2-[(2-aminoethyl)amino]-; trans-1,10-dimethyl-trans-9-

decalinol. A clear disparity observed between the biomolecules expressed in the interaction of *A. faecalis* SASBG203 with *C. orbiculare* when compared with individual organisms alone. This pattern was well represented in the heat map (Figure. 5).

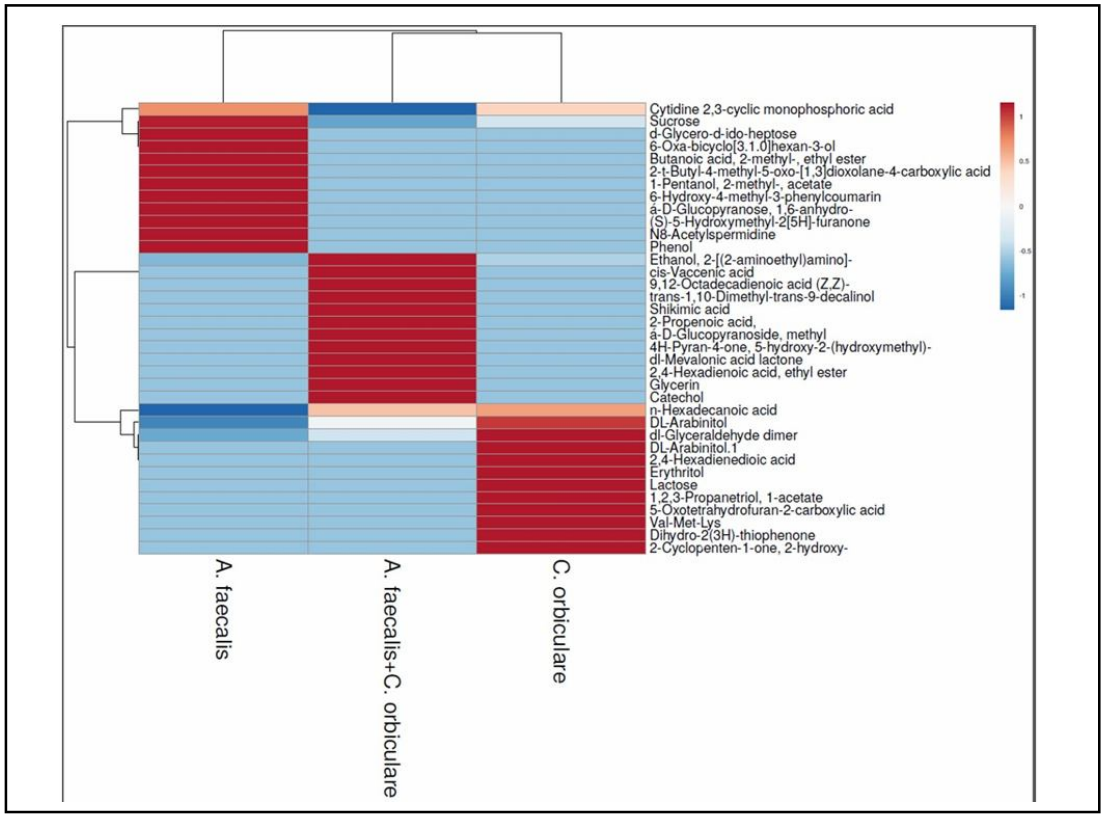

Figure 5: Heat map of soluble metabolitcs from individual growth of *A. faecalis* strain SASBG203, *C. orbiculare* and their interaction in dual culture.

3.5 Pathway topology analysis and pathway impacts

The pathway topology and impact values (cumulative percentage from the metabolite nodes) of interaction of *A. faecalis* strain SASBG203 and *C. orbiculare* revealed six key metabolic pathways, *viz.*, biosynthesis of unsaturated fatty acids, glycerolipid metabolism, phenylalanine, tyrosine and tryptophan biosynthesis, glycolysis/gluconeogenesis, fatty acid degradation and fatty acid biosynthesis (Table.2).

Table 2: Pathways identified using METABOANALYST 5.0 in the interaction of *A. faecalis* strain SASBG203 with *C. orbiculare* in dual culture assay

S.No.	Pathway	RawP	FDR
1.	Biosynthesis of unsaturated fatty acids	2.58E-02	1.00E+00
2.	Glycerolipid metabolism	1.49E-01	1.00E+00
3.	Phenylalanine, tyrosine and tryptophan biosynthesis	2.15E-01	1.00E+00
4.	Glycolysis/gluconeogenesis	2.42E-01	1.00E+00
5.	Fatty acid degradation	2.94E-01	1.00E+00
6.	Fatty acid biosynthesis	3.95E-01	1.00E+00

The pathways, glycerolipid metabolism and Phenylalanine, tyrosine and tryptophan biosynthesis with impact values of 0.29 and 0.08 were unique in the interaction of *A. faecalis* strain SASBG203 with the fungus (Figure 6).

3.6 Metabolite set enrichment analysis (MESA)

Metabolite set enrichment analysis was performed to identify biologically meaningful patterns that are significantly enriched in quantitative metabolomic data. Over representation, analysis (ORA) was implemented using a hypergeometric test to evaluate whether a particular metabolite set is represented more than expected by chance within the given compound list based on one tailed *p* values. The pathways of the following metabolism were revealed during the dual culture interaction, *viz.*, bile acid biosynthesis, steroid biosynthesis, fatty acid metabolism, galactose metabolism, fatty acid biosynthesis, fatty acid elongation in mitochondria, fructose and mannose degradation, ethanol degradation, alpha linolenic acid and linoleic acid metabolism and glycerolipid metabolism. In general, bile acid biosynthesis, steroid biosynthesis and fatty acid metabolism were over represented in the dual culture of *A. faecalis* strain SASBG203 and *C. orbiculare* with *p*-value > 0.05 (Figure 7).

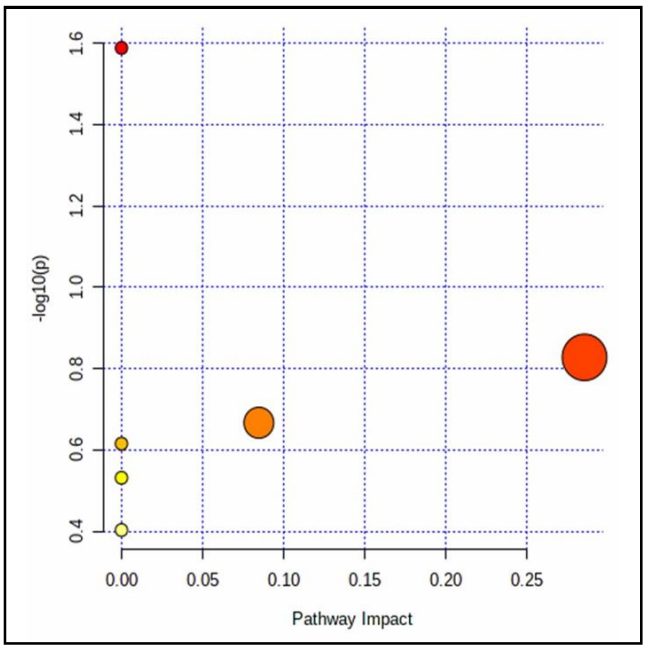

Figure 6: Impact of biomolecules identified in the interaction of *A. faecalis* strain SASBG203 and *C. orbiculare* in various metabolic pathways identified using METABO ANALYST 5.0 software.

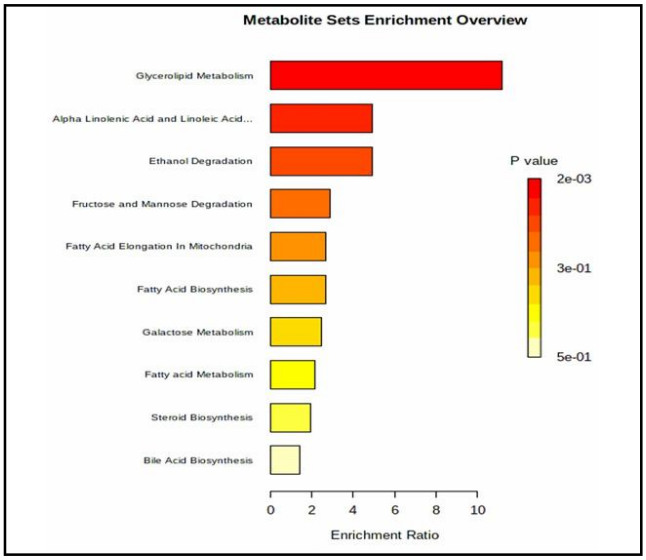

Figure 7: Over representation analysis of selected biomolecules in interaction of *A. faecalis* strain SASBG203 and *C. orbiculare* compared to the pathways of *Saccharomyces cerevisiae*, a typical fungal organism.

4. Discussion

In this study, bacterial endophytes were tested for their efficacy *in vitro* against cucurbit stem splitting pathogen, *C. orbiculare* in dual culture assays. One of the antagonistic bacterial endophytes, *A. faecalis* strain SASBG203 exhibited significant inhibitory effect on fungal growth to the tune of 71.08 per cent. Nowadays, indiscriminate use of plant protection chemicals has increased many folds which can cause health hazards; on the other hand, biological control of the plant pathogens is a healthier solution where microorganisms or their products will be deployed against plant pathogens (Bahadir Torun *et al.*, 2018; Ved Ratan *et al.*, 2018). The endophyte, *A. faecalis* strain SASBG203 was positive for production of IAA, amylase, acetoin, nitrogen, phosphate, cellulase, protease, and for 2, 3, butane diol denoting its ability to promote plant growth at large. Similarly, Dilfuza Jabborova *et al.* (2020) reported four bacterial endophytes (GS2, GS5, GS8, and GS10) which can solubilize phosphate, produce IAA, siderophore, lipase, protease, and cellulose besides showing antifungal activity against *Fusarium. sporotrichiodes* 404, *F. globosum* 905, *F. graminearium* 611, *F. oxysporum* 328, *F. culmorum, F. solani, F. proliferatum* 516. These endophytes were inherently resistant to climatic extremities and can get well adapted in the host plant. Several previous studies indicated the beneficial effects of endophytes (Singh *et al.*, 2013; Ali and Rante, 2018). The molecular identity of the effective endophyte *A. faecalis* strain SASBG203 was established through amplification of 16s rRNA region universal primers 27 F and 1492 R. Phylogenic lineages were drawn through MEGA X software utilizing the retrieved sequences from NCBI database.

The soluble metabolites from the individual growth of *A. faecalis* strain SASBG203, from *C. orbiculare* and from their interaction were profiled through gas chromatography and mass spectrometry (GC-MS). The biomolecules expressed as peaks in chromatogram were searched against databases like NIST library and PubChem to arrive at their identity and functionality. The biomolecules specifically expressed in the interaction of *A. faecalis* strain SASBG203 and *C. orbiculare* were viewed as potent molecules with biocontrol activity, *viz.*: (1) Shikimic acid is a key intermediate in defence related pathways and precursor of amino acids like tryptophan, tyrosine and phenylalanine production pathways in plants, fungi and bacteria (Mohammed *et al.*, 2018). Chorismate, the end product of the shikimate pathway is the origin of the isochorismate pathway and the phenylalanine ammonia lyase pathway for production of salicylic acid, an important signal in defence related pathways of plants (Dempsey *et al.* 2011), (2) 2-Propenoic acid reported in antifungal action (Gomes *et al.*, 2018), (3) dl-Mevalonic acid lactone was previously reported from fungal endophyte in *Zingiber officinale* and suppressed the soft rot pathogen, *Pythium* spp. (Anisha and Radhakrishnan, 2017); (4) 4H-pyran-4-one, 5-hydroxy-2-(hydroxymethyl)-, was known to be antioxidative and cytotoxic (Madhuree Kumari *et al.*, 2018).3-O-methylfunicone (OMF), a derivativeof 4-pyrone isolated from species of talaromyces (Nicoletti *et al.*, 2009), exhibited notable fungitoxic and antitumour properties (De Stefano *et al.*, 1999; Nicoletti *et al.*, 2014); (5) 2,4-Hexadienoic acid, ethyl ester (Madhuree Kumari *et al.*, 2018); (6) Glycerin. Foliar application of glycerol suppressed *Phytophthora capsici* by enhancing glycerol-3-phosphate and reactive oxygen species production in cacao (Yufan Zhang *et al.*, 2015) and it was also involved in plant defense responses (Akshaya *et al.*, 2021); (7) Catechol; it is involved in the catabolism of salicylic acid, an important hormone in plant immune reactions (Kristen *et al.*, 2020).(8) cis-Vaccenic acid; increased concentration of cis-vaccenic acid (18:1omega7c) in fatty acid profiles of antagonists was associated with suppression of *Rhizoctonia* soft rot in cucumber roots (Tunlid *et al.*, 1989); (9) 9,12-octadecadienoic acid (Z,Z)-; Ali *et al.* (2017) attributed the antifungal activity of root exudates of *Chenopodium album* to

presence of octadecadienoic acid in methanolic extracts. Gayathri *et al.* (2021) reported antifungal and antibacterial properties of n-hexadecanoic acid, and (10) Ethanol, 2-[(2-aminoethyl)amino].

Antimicrobial properties of metabolites from *A. faecalis* cultures were previously discussed by many authors. *A. faecalis* is seen as potential biocontrol agent against *E. psidii* with the presence of N-formylmaleamic acid, oleamide and D-1-piperideine-2-carboxylic acid found in the diethyl ether extracts (Khim *et al.*, 2019). *A. faecalis* strain AD15 produced hydroxylamine at maximum yields of 33.3 ± 1.7 mg/l after 16 h cultivation in LB medium and 19.0 ± 0.44 mg/l after 19 h of cultivation in synthetic medium. Hydroxylamine at minimum concentrations of 4.20 ± 0.98 and 16.5 ± 0.67 mg/l was inhibitory to the cyclamen pathogens *Pantoea agglomerans* and *Colletotrichum gloeosporioides* respectively (Shin-Ichiro Yokoyama, 2013). *A. faecalis* No. 4 was introduced as a fungistatic bacterium, because it produces hydroxylamine (Honda *et al.*, 1998; Joo *et al.*, 2005). *Alcaligenes* sp. YL-02632S produced polyketides kalimantacin/batumin that was bactericidal for *Staphylococcus aureus* (Kamigiri *et al.*, 1996; Tokunaga *et al.*, 1996). Similarly, Zou *et al.* (2007) reported *A. faecalis* MHS033 and MHS013 produced fungistatic VOCs, such as methanamine, 1-butanamine, and benzaldehyde. Kavroulakis *et al.* (2010) reported that *Alcaligenes* sp. AE1.16 produced glucanase, chitinase and hydrogen cyanide and can be used as biocontrol agents.

GC-MS analysis of crude ethyl acetate of *A. faecalis* (CEAF) was reported to have compounds related to antimicrobial, antifungal, antioxidant, pesticide, metabolism, toxicity, anticancer and corrosion inhibition activities. The crude ethyl acetate extract of *A. faecalis* showed the ability to inhibit sulphate reducing bacterial (SRB) growth in petroleum reservoirs (Ali Abd Sharad *et al.*, 2016). Shaojie Shan *et al.* (2018) reported the volatiles of symbiotic bacterium, *A. faecalis* from entomopathogenic nematode *Oscheium* spp. as inhibitory to the growth of plant pathogenic fungus, *Botrytis cinerea* and entomo pathogenic fungi *Mucor circinelloides*, *M. racemosus* and *Rhizomucor variabilis*. The inhibitory effect was attributed to dimethyl disulfide (DMDS) in the volatiles. Antifungal properties of DMDS were previously discussed by Pecchia *et al.* (2017). Siderophore rich culture broth, siderophore rich supernatant and purified siderophore preparation from *A. faecalis* exerted antifungal activity against *Aspergillus niger* NCIM 1025, *A. flavus* NCIM, 650, *Fusarium oxysporum* NCIM 1008 and *Alternaria alternata*, IARI 715 (Sayyad *et al.* 2008). Sayed E. El-Sayed *et al.* (2020) first isolated and reported octadecyl 3-(3, 5-di-tert-butyl-4-hydroxyphenyl) propanoate from the culture broth of *A. faecalis* MT332429 with a promising antifungal activity along with its optimized production through response surface methodology (RSM). Gong *et al.* (2019) through gas chromatography tandem mass spectrometry revealed dimethyl disulfide (DMDS) and methyl isovalerate (MI) were two abundant compounds in the volatile profiles of *A. faecalis* N1-4. DMDS prevented the conidia germination and mycelial growth of *Aspergillus flavus* at 50 and 100 µl/l, respectively and the effective concentration for MI against *Aspergillus flavus* was 200 µl/l.

Furthermore, biosynthetic pathway topology and pathway impact assessment revealed glycerolipid metabolism and phenylalanine, tyrosine and tryptophan biosynthesis with impact values of 0.29 and 0.08 were unique in the interaction of *A. faecalis* strain SASBG203 with the fungus. Dong *et al.* (2019) reported the endophytes *Arthrobacter endophyticus* and *Nocardiopsis alba* promoted the pathways such as carotenoid biosynthesis, phenylalanine metabolism, phenylpropanoid biosynthesis, glycerolipid metabolism, and nitrogen metabolism which played a crucial role in enhancing the salt stress tolerance of *A. thaliana*. Sreedharan *et al.* (2021) unravelled the induction of amino sugar metabolism during suppression of *Macrophomina phaseolina* by a fungal endophyte *Trichoderma longibrachiatum* EF5.The over representation analysis of soluble biomolecules against human metabolome suggested that they are highly metabolized in bile acid biosynthesis, steroid biosynthesis and fatty acid metabolism.

5. Conclusion

In this study, a total of 15 bacterial endophytes from bottle gourd were isolated and tested for their effectiveness against bottle gourd stem splitting pathogen, *C. orbiculare* in dual confrontation assays. One of the effective endophytes, was identified as *A. faecalis* by employing 16s rRNA universal primers 27 F and 1492 R. Further, metabolic profiling of biomolecules by gas chromatography and mass spectrometry revealed various compounds with antifungal activity. The prominent compounds were, *viz*., shikimic acid, a key intermediate in defense related pathways and precursor of amino acids like tryptophan, tyrosine and phenylalanine production pathways in plants, fungi and bacteria; 2-propenoic acid and dl-mevalonic acid lactone with antifungal properties; 4-pyrone with fungitoxic and antitumor properties; glycerin known to produce ROS in defense reactions and catechol which was reported in catabolism of salicylic acid. These soluble metabolites were assessed for pathway impact in typical fungal metabolome of *Saccharomyces cerevisiae* and metabolite set enrichment analysis were conducted to arrive at involvement in specific physiological pathways.

To our knowledge, this is the first report of bacterial endophyte, *A. faecalis*as biocontrol agent against *C. orbiculare* and the soluble biomolecules produced therein. Further, studies are required to determine specific effects of these biomolecules against the test pathogen and to develop suitable organic based formulation for spray on cucurbits at large.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

References

Akshaya, S.B.; Krishnamoorthy, A.S.; Sangeetha, C.; Nakkee-ran, S. and Thiribhuvanamala, G. (2021). Investigation on antifungal metabolites of Chinese caterpillar fungus *Ophiocordyceps sinensis* (Berk.) against wilt causing pathogen, *Fusarium* spp. Ann. Phytomed. **10**(1): 195-201, http://dx.doi.org/10.21276/ap.2021. 10.1.20

Ali, A. and Rante, H. (2018). Screening of endophytic bacteria producing antifungal isolated from Indonesia medicinal plant, java ginseng (*Talinum triangulare*) (jacq.) willd. Intl. J. Pharm. and Pharmaceutl., **10**(6):152-158.

Ali, A. S.; Gires, U.; Fathul, K. S. and Asmat, A. (2016). Antimicrobial activity and determination of bioactive components from marine *Alcaligenes faecalis* extract against a sulfate-reducing bacteria. American Institute of Physics Conference Proceedings **1784**,020010.https://doi: 10.1063/1.4966720.

Alia, A.; Javaida and Shoaib (2017). GC-MS analysis and antifungal activity of methanolic root extract of *Chenopodium album* against *Sclerotium rolfsii*. Planta Daninha, **35**. https://doi.org/10.1590/S0100-83582017350100046

Anisha, C. and Radhakrishnan, E.K. (2017). Metabolite analysis of endophytic fungi from cultivars of *Zingiber officinale* Rose. Identifies myriad of bioactive compounds including tyrosol. 3 Biotech. **7.** (Article number. 146). https://doi.org/10.1007/s13205-017-0768-8

Bahadir Torun, H.; Halil, B.; Zeynep, E. and Esin, P. C. (2018). Antifungal activities of *Urtica dioica* L., *Sinapis arvensis* L. and *Apium graveolens* Mill. leaves on *Botrytis cinerea* Pers. Ann. Phytomed. **7**(2):94-97. https://doi.org/10.21276/ap.2018.7.2.15

Cawoy, H.; Mariutto, M.; Guillaume, H.; Fisher, D.; Vasilyeva, N. and Thonart. P (2014). Plant defence stimulation by natural isolates of Bacillus depends on efficient surfactin production. MPMI., **27:**(87-100). https://doi.org/10.1094/MPMI-09-13-02-62-R

Chernin, L.S.; Ismailov, Z.F.; Haran, S. and Chet, I. (1995) Chitinolytic *Enterobacter agglomerans* antagonistic to fungal plan pathogens. Appl. Env. Microb., **61**:1720-1726.

De Stefano, S.; Nicoletti, R.; Milone, A. and Zambardino, S. (1999). 3-O-Methylfunicone, a fungitoxic metabolite produced by the fungus *Penicillium pinophilum*. Phytochemistry, **52:**1399-1401.

Dempsey, D'Maris, A.; Vlot, A. C.; Wildermuth; Mary, C.; Klessig and Daniel, F. (2011). Salicylic Acid Biosynthesis and Metabolism. The Arabidopsis Book, 2011(9) Published By: The American Society of Plant Biologists URL: https://doi.org/10.1199/tab.0156.

Denizci, A.A.; Kazan, D.; Abeln, E.C.A. and Erarslan, A. (2004). Newly isolated *Bacillus clausii* GMBAE42: An alkaline protease producer capable to grow under highly alkaline conditions. J. Appl. Microb., **96:** 320-327.

Dilfuza Jabborova.; Kannepalli, A.; Mohina, F.; Khurshid, S.; Dilbar, K., Zafarjon, J. and Sayyed, R. Z. (2020). Isolation and characterization of endophytic bacteria from ginger (*Zingiber officinale* Rosc.). Ann. Phytomed., **9**(1):116-121. http://dx.doi.org/10.21276/ap.2020.9.1.14.

Digar Singh.; Anita Sharma. and Gurvinder Kaur Sain. I (2013). Biochemical and molecular characterisation of the bacterial endophytes from native sugarcane varieties of Himalayan region. 3 Biotech. **3**:205-212. https://doi.org/10.1007/s13205-012-0084-2

Doyle,J. J. and Doyle, J. L. (1990). Isolation of plant DNA from fresh tissue. Focus, **12**(1):13-15.

Gayathiri, M.; Thiribhuvanamala, G.; Krishnamoorthy, A.S.; Haripriya, S.; Akshaya, S.B. and Arumukapravin, I. (2021). Characterization of antimicrobial metabolites from medicinal mushrooms against Mango anthracnose pathogen *Colletotrichum gloeosporioides* (Penz.) Sacc. Ann. Phytomed., **10**(1):185-194, http://dx.doi.org/10.21276/ap.2021.10.1.19

Gomes, A. A. M.; Danilo, B. P.; Zenilda del. Cardeal.; Helvécio, C. Menezes.; Marisa, V. De Queiroz. and Olinto, L. Pereira (2018). *Simplicillium coffeanum*, a new endophytic species from Brazilian coffee plants, emitting antimicrobial volatiles. Phytotoxa., **333**(2):188-198.https://doi.org/10.11646/phytotaxa.333.2.2.

Gong, A. D.; Wu, N. N.; Kong, X.W.; Zhang, Y, M.; Hu, M. J.; Gong, S.J.; Dong, F.Y.; Wang, J.H.; Zhao, Z.Y. and Liao, Y.C. (2019). Inhibitory effect of volatiles emitted from *Alcaligenes faecalis* N1-4 on *Aspergillus flavus* and aflatoxins in storage. Frontiers in Microbiology, **10**:1419. https://doi: 10.3389/fmicb.2019.01419.

Hansen, P.A. (1930). The detection of ammonia production by bacteria in agar slants. J. Bacteriology. American Society of Microbiology, pp: 223-229.

Honda, N.; Hirai, M.; Ano, T. and Shoda, M. (1998) Antifungal effect of a heterotrophic nitrifier *Alcaligenes faecalis*. Biotech. Lett, **20**:703-705.

Irma, M. (2018). Identification of endophytic bacterial isolated from oil palm plants with antifungal acitvity against *Ganoderma boninense*. Pharma. Clini. Pharma. Res., **3**(2):41-49. https://doi:10.15416/pcpr.v3i2.20005

Joo, H. S.; Hirai, M. and Shoda, M. (2005). Characteristics of ammonium removal by heterotrophic nitrification-aerobic denitrification by *Alcaligenes faecalis* No. 4. J. Biosci. Bioeng., **100:**184-191.

Fasim, F.; Ahmed, N.; Parsons, R. and Gadd, G.M. (2002). Stabilization of zinc salts by a bacterium isolated from the air environment of a tannery. FEMS Mirobiol. Lett., **213**:1-6.

Kamigiri, K.; Suzuki, Y.; Shibazaki, M.; Morioka, M. and Suzuki, K. (1996). Kalimantacins A, B and C, novel antibiotics from *Alcaligenes* sp. YL-02632S I. Taxonomy, fermentation, isolation and biological properties. J. Antibiotics, **49:**136-139.

Kavroulakis, N.; Ntougias, S.; Besi, M. I.; Katsou, P.; Damaskinou, A.; Ehaliotis, C.; Zervakis, G. I. and Papadopoulou, K. K. (2010). Antagonistic bacteria of composted agro-industrial residues exhibit antibiosis against soil-borne fungal plant pathogens and protection of tomato plants from *Fusarium oxysporum*f. sp. *radicis-lycopersici*. Plant Soil, **333:**233-247.

Khim, P. C.; Racheal, K.S.L. and Jualang, A. G.(2019). Bioactivity of *Alcaligenes faecalis* and *Lecanicillium* sp. isolated from crocker range of Sabah against *Erwinia psidii* of papaya dieback disease. Malaysian J. Microb., **15**(5):387-393.

Kristen, V.G.; Taylor, F. and Tariq, A. A.(2020). Evidence from stable-isotope labelling that catechol is an intermediate in salicylic acid catabolism in the flowers of *Silene latifolia* (white campion). Planta, **252**(1):3. https://doi: 10.1007/s00425-020-03410-5.

Madhuree, K.;Sidhartha, T.;Ankur, S. and Jayabaskaran, C(2018). Antiproliferative and antioxidative bioactive compounds in extracts of marine-derived endophytic fungus *Talaromycespurpu reogenus*. Fron. Microb., **9**:1777. https://doi: 10.3389/fmicb.2018. 01777.

Mishra, I.G.; Tripathi, N, and Tiwari, S. (2014). A simple and rapid DNA extraction protocol for filamentous fungi efficient for molecular studies. Indian J. Biotechnol., **13**:536-539.

Mohammed, A. K.; Alberto, M. and Maged, E. M.(2018). Rational discovery of (+) (S) abscisic acid as a potential antifungal agent: A repurposing Approach. Sci. Reports, **8**:8565. https://doi: 10.1038/s41598-018-26998-x.

Muhammad Zahoor.; Muhammad Ikram.; Nausheen Nazir.; Sumaira Naz.; Gaber, E.S.B.; Abdul W. K.; Michal, T. and Ahmed, K., (2021). A comprehensive review on the medicinal importance: Biological and therapeutic efficacy of *Lagenaria siceraria* (Mol.) (Bottle gourd) standley. Fruit. Curr. Topics Med. Chem., **21**(20):1788-1803. https://doi: 10.2174/1568026621666210701124628.

Nicoletti, R.; Scognamiglio, M. and Fiorentino, A. (2014). Structural and bioactive properties of 3-O-methylfunicone. Mini Reviews Medical Chemistry, **14**:1043-1047.

Nicoletti, R.; Manzo, E. and Ciavatta. M. L. (2009). Occurrence and bioactivities of funicone-related compounds. Intern. J. Mol. Sci., **10:**1430-1444.

Nuraini, M. N. and Latiffah, Z. (2018). Identification and characterization of *Colletotrichum* spp. associated with chili anthracnose in peninsular Malaysia. Euro. J. Pl. Path., **151**:961-973.

Pecchia, S.; Franceschini, A.; Santori, A.; Vannacci, G. and Myrta, A. (2017). Efficacy of dimethyl disulfide (DMDS) for the control of *Chrysanthemum verticillium* wilt in Italy. Crop Protection, **93**:28-32.

Saha, A.; Das, S.; Chakraborty, P.; Saha, B. and Saha, D. (2002). Two new bottle gourd fruit rot causing pathogens from Sub - Himalayan West Bengal. Int. J. Agri. Biotech., **12**(4):707-719.

Saitou, N. and Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. and Evol., **4**:406-425.

Sayed, E. E.S.; Neveen, A.; Abdelaziz.; Ghadir, S. E.H. and Khaled M. A.(2020). Octadecyl 3-(3, 5-di-tert-butyl-4-hydroxyphenyl) propanoate, an antifungal metabolite of *Alcaligenes faecalis* strain MT332429 optimized through response surface methodology. Appl. Micro. Biotech., **104**:10755-10768. https://doi.org/10.1007/s00253-020-10962-9

Sayyed, R. Z.; Patil, A. S.; Gangurde, N. S.; Bhamre, H. M.; Joshi, S. A. and Fulpagare, U. G. (2008). Siderophore producing *A. faecalis*: A potent biofungicide for biocontrol of groundnut phytopathogens. Proceeding of ISBT 2008 - Oral Papers (28th-30th Dec 2008):411-413.

Shanmugaraj, C.; Archana, A. and Vinay, K. (2021). Determination of fermentation pathway by methyl red and voges proskauer (MRVP) test. AgriCos e-Newsletter, **2**(11):https://www.researchgate.net/publication/356128142.

Shaojie Shan.; Wenwu Wang.; Chunxu Song.; Minggang Wang.; Bingjiao Sun.; Yang Li.; Yaqi Fu.; Xinghui Gu.; Weibin Ruan. and Sergio Rasmann (2019). The symbiotic bacteria *Alcaligenes faecalis* of the entomopathogenic nematodes *Oscheius* spp. exhibit potential biocontrol of plant- and entomopathogenic fungi. Micro. Biotech., **12**(3):459-471. https://doi.org/10.1111/1751-7915.13365

Shin-ichiroYokohama.; Yoshitomi Adachi.; ShichiAsakura. and Erina Kohyama (2013). Characterization of *Alcaligenes feacalis* strain AD15 indicating biocontrol activity against plant pathogens. J. Gen. Appl. Microb., **59**:89-95.

Singh, R.; Kumar, A.; Singh, M. and Pandey, K.D. (2013). Effect of salt stress on endophytic bacteria isolated from the root of *Momordica charantia*. In: Indian Society of Vegetable Science, National Symposium on Abiotic and Biotic Stress Management in Vegetable Crops.

Sridharan. A.P.; Sugitha, T.; Karthikeyan. G.; Nakkeeran, S. and Sivakumar, U. (2021). Metabolites of *Trichoderma longibrachiatum* EF5 inhibits soil borne pathogen, *Macrophomina phaseolina* by triggering amino sugar metabolism. Microbial Pathogenesis, **150**:104-714. https://doi.org/10.1016/j.micpath.2020.104714.

Sriskandarajah, S.; Kennedy, I.R.; Daiguan, Y.U. and Tchan, Y.T. (1993). Effects of plant growth regulators on acetylene-reducing associations between *Azospirillum brasilense* and wheat. Plant Soil, **153**:165-178.

Tamura, K.; Peterson, D.; Nicholas Peterson.; Glen Stecher.; Masatoshi Nei. and Sudhir Kumar. (2011). MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol., **10**:2731-2739. https://www.doi.org.10.1093/molbev/msr121.

Tokunaga, T.; Kamigiri, K.; Orita, M.; Nishikawa, T.; Shimizu, M. and Kaniwa, H. (1996). Kalimantacins A, B and C, novel antibiotics produced by *Alcaligenes* sp. YL-02632S II. Physicochemical properties and structure elucidation. J.Antibiotics, **49**:140-144.

Tunlid,A.; Hoitink, H. A.; Low, C. and White, D.C.(1989). Characterization of bacteria that suppress rhizoctonia damping-off in bark compost media by analysis of fatty acid biomarkers. App. Envi. Microb., **55**(6):1368-1374. https://doi: 10.1128/aem.55.6.1368-1374.1989.

Ved Ratan.; Supriya, D.; Mukesh, S.; Shubha, T.; Abhishek, M.; Srivastava, Y.K. and. Srivastava, D.K. (2018). Computational structure prediction and analyze active ligand binding site of defense and lytic enzymes of *Trichoderma harzianum*. Ann. Phytomed., **7**(2):143-160. https://doi.org/10.21276/ap.2018.7.2.23.

Watanabe, K.; Kodama, Y. and Harayama, S. (2001). Design and evaluation of PCR primers to amplify bacterial 16S ribosomal DNA fragments used for community fingerprinting. J. Microb. Methods, **44**:253-262 https://doi.org/10.1111/jam.14818.

White, T.J.; Bruns, T.; Lee, S.J.W.T. and Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics In: Innis MA Gelfand DH Sninsky JJ and White TJ (ed) PCR protocols: a guide to methods and applications. pp:315-22 DOI:10.1016/b978-0-12-372180-8.50042-1.

Yufan Zhang.; Philip Smith.; Siela, N. M. and Mark, J. G.(2015). Application of glycerol as a foliar spray activates the defence response and enhances disease resistance of *Theobroma cacao*. Mol. Pl. Path., **16**(1):27-37.https://doi: 10.1111/mpp.12158.

Zhan-Bin,S.; Yuan, X.F.; Zhang, H.; Wu,L. F.; Liang, C. and Feng, Y. J. (2013). Isolation, screening and identification of antagonistic downy mildew, endophytic bacteria fromcucumber. Euro. J. Pl. Path., **137**:847-857.

Zhou-YanDong.;Manik, P.N.R.;Hong-FeiWang.;Bao-ZhuFang.; Yong-HongLiu.; LiL.M.X. And Wen-JunLi., (2019). Transcriptomic analysis of two endophytes involved in enhancing salt stress ability of *Arabidopsis thaliana*. Science of the Total Environment, **686**:107-117.https://doi.org/10.1016/j.scitotenv.2019.05.483.

Zou, C.S.; Mo, M.H.; Gu, Y.Q.; Zhou, J.P. and Zhang, K.Q. (2007). Possible contributions of volatile-producing bacteria to soil fungistasis. Soil Bio. Biochem., **39**:2371-2379.

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