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Study of Endophytes as Biocontrol Agents *vis-a-vis* their Compatibility to Fungicides in Grapes

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ABSTRACT

The present study was conducted at ICAR-National Research Centre for Grapes in Pune, Maharashtra, India during 2021–2022 to study endophytic bacteria as biocontrol agents *vis-a-vis* their compatibility to fungicides in grapes. Eighteen endophytic bacteria were isolated from nine varieties of grapevine (*Vitis vinifera* L.) viz. Manjari Naveen (MN), Nanasaheb Purple (NP), Thompson Seedless (TS), Crimson Seedless (CS), Manik Chaman (MC), Maruti Seedless (MS), Fantasy Seedless (FS) and 2A Clone (2A). The distinct colonies were selected, sub-cultured, purified and subjected to analysis of macroscopic and microscopic features followed by screening of bacterial isolates for plant growth-promoting (PGP) traits, extracellular hydrolytic enzyme production, and abiotic stress tolerance assay. Eighteen diverse endophytic bacteria were found to be Gram positive to Gram negative and with white, whitish yellow and whitish red colonies. Different biochemical tests which were needful for the identification of the bacteria were performed. Antibiotic sensitivity and antifungal assays were conducted with five antibiotics and pathogenic fungi *Colletotrichum gloeosporioides* respectively. Results of dual culture plate assay for antimicrobial activity revealed that 10 isolates showed significant growth inhibition of the test pathogen and were selected for the compatibility studies. The results highlighted that most of the isolates were highly compatible with all the fungicides used for powdery mildew, downy mildew, anthracnose and bacterial leaf spot. The work indicated the importance of the endophytic bacteria which can be used as promising biocontrol agents for grapevine disease management.

KEYWORDS: Antagonism, biochemical characterization, biocontrol agents, endophytes, fungicide, grape

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1. INTRODUCTION

In India, grape (*Vitis vinifera* L.) is an important fruit crop of high economic value with an export of 267,950 t grown over approximately from 162 thousand ha during 2021–22 (Anonymous, 2021). Globally the annual production of grape is approximately 75 mt, with the largest production in Europe (about 41%), followed by Asia (29%) and the America (21%) (Colombo et al., 2019, Unusan, 2020, Zhou et al., 2022). It can be consumed fresh as table grapes or used to prepare wine, jam, grape juice, grape seed oil, jelly, raisins and vinegar. Polyphenols such as antioxidants and other secondary metabolites are abundant in grapes having several health benefits (Sabra et al., 2021). The phytochemicals extracted from the seed, skin and grape juice includes carotenoids, melatonin, resveratrol and phenolics, which are used for various commercial processes (Yang and Xiao, 2013, Ono et al., 2020).

Endophytes are a group of microorganisms that promote plant growth while residing within plant tissue without harming the host (Yadav, 2018, White et al., 2019). These microbes have distinct beneficial properties which include substantial disease resistance, alleviation of soil saturation and drought stress and increased competition which, in turn benefit the host plant against various biotic and abiotic stresses (Elmagzob et al., 2019, Shahid et al., 2022). Endophytes of grapevine are associated with berry as well as leaves (phyllosphere) (Martins et al., 2013, Vionnet et al., 2018). Their colonization in the grape tissue can provide intensified immunity and safeguard the entire plant from different pathogens and insects by producing secondary metabolites like phytoalexins, biocides like hydrogen cyanide (HCN) and antibiotics (Pacifico et al., 2019, Wu et al., 2021).

Grapevines are highly susceptible to different pathogens such as *Erysiphe necator, Plasmopara viticola*, *Colletotrichum gloeosporioides, Xanthomonas citri* pv *viticola* etc. due to which severe economic losses had been seen over a period of time (Armijo et al., 2016, Pacifico et al., 2019, Rumbaugh et al., 2021). Fungicides are the cornerstones of disease management but their excessive application had resulted in a series of environmental and ecological problems which seriously affect the sustainable development of agriculture (Chatterjee et al., 2016, De Silva et al., 2019). It is reported that the grape exporters from Maharashtra met a loss of ₹ 250 crore after being rejected their consignments by the European Union countries due to a chemical residue of chlormequat chloride in 2010 (Anonymous, 2010). Moreover, fungicide-resistant *Colletotrichum gloeosporioides* (Penzig) Penzig and Saccardo, which caused anthracnose to grapevine had emerged India and Japanese vineyards (Hamaoka et al., 2021). Narkar et al. (2012) reported *Colletotrichum gloeosporioides* being resistant to carbendazim. Thus, it was imperative to find an alternative measure to suppress the onslaught of fungal diseases. Microorganisms belonging to the genera *Bacillus*, *Streptomyces*, *Pseudomonas*, *Burkholderia* and *Agrobacterium* had been studied and used as the potential biocontrol agents (Mabrouk et al., 2018, Chandra et al., 2018, Le et al., 2022) due to their antifungal and antibacterial activity against phytopathogens (Maksimova et al., 2011, Kohl et al., 2019, Rat et al., 2021).

Despite adequate knowledge on potential plant growth-promoting bacterial endophytes in grapes, their investigation in Indian perspective in the domains of characterisation, bio-control potential against grape pathogens and compatibility with fungicide are yet to be accomplished. The present study was aimed to fill the aforementioned information gap by focussing on the endophytic bacteria isolated from the leaf of different varieties of *Vitis vinifera*.

2. MATERIALS AND METHODS

2.1. Sample collection

Fresh and healthy leaves were collected from different grape vine varieties including three white varieties viz. Manjari Naveen (MN), Thomson Seedless (TS), Manik Chaman (MC) and seven coloured varieties viz. Nanasaheb Purple (NP), Crimson Seedless (CS), Maruti Seedless (MS), Fantasy Seedless (FS), 2A Clone (2A), Sarita Seedless (SS) and Manjari Shyama (MSH) grown in the experimental plots of ICAR - National Research Centre for Grapes, Pune, Maharashtra, India (latitude 18.31°N, longitude 73.55° E and 559 m above mean sea level) during 2021–2022.

2.2. Isolation of endophytes

The isolation of endophytic bacteria from grapevine leaves were carried out as per protocol described by de Oliveira Costa et al. (2012). The selected colonies were sub-cultured and purified following the method by Shah et al. (2022) with minor modifications. Macroscopic and microscopic features of the isolated bacterial colonies were assessed (Bartholomew and Mittwer, 1952).

2.2.1. Biochemical characterization of purified bacterial isolates

Biochemical characterization of bacteria was done by performing different biochemical assays.

2.2.1.1. Potassium hydroxide (KOH) test

Bacterial colony was mixed with 1–2 drops of 3% KOH solution on slide. When inoculation loop was raised after few seconds of stirring, formation of thread like structure was considered as positive for the test indicating that the particular isolate was Gram negative and vice versa (Suslow et al., 1982).

2.2.1.2. Sugar utilization test

The bacterial isolates were inoculated in phenol-red nutrient

broth containing different sugars (1%) viz. dextrose, glucose, sucrose and fructose separately. The broths were incubated at 37°C for 24 h. Colour change in the medium from red to yellow was considered positive test for carbohydrate utilization (Pal et al., 2012).

2.2.1.3. Catalase, urea hydrolysis and gelatin liquefication test

Catalase activity, urease activity and liquefication of gelatin was performed using protocol of Vashist et al. (2013) with slight modifications.

2.3. Screening of bacterial isolates for plant growth-promoting (PGP) traits and extracellular hydrolytic enzymes production

2.3.1. Indole production test

Four ml of 1% tryptone broth was dispensed in test tube and autoclaved at 15 psi pressure and 121°C for 15 m. Each tube was inoculated with loopful of test organism and an uninoculated tube was taken as a control. After incubation at 37°C for 48 h, 1 ml of Kovac's reagent was added in each tube and then the test tubes were vortexed vigorously for 1 minutes. Formation of cherry-red coloured ring indicated the positive reaction (Vashist et al., 2013).

2.3.2. Hydrogen cyanide (HCN) production test

Bacteria were grown on nutrient agar medium supplemented with glycine (4.4 g l^{-1}). Whatman's filter paper number 1(HiMedia Laboratories Pvt. Ltd. India) dipped in 0.5% (w/v) picric acid solution was placed on the lid of petri dish and was sealed with parafilm and incubated for 7 days at 37°C in an incubator. Colour change of filter paper from yellow to reddish brown was noted as positive for HCN production.

2.3.3. Ammonia production

Peptone water medium was used for ammonia production assay by dispensing 10 ml media in each test tube. Sterilized peptone water was inoculated with a loopful of bacteria and an uninoculated tube was treated as control. Tubes were incubated for 2 days at 37°C in an incubator. Brownish yellow colour development in the test tubes was recorded as a positive reaction.

2.3.4. Enzyme hydrolysis tests

The tests included (1) starch hydrolysis on starch plates (Claus, 1988); (2) Lipid hydrolysis using Tributyrin agar plates (Lusty and Doudorof, 1966); and (3) proteolysis on skim milk agar plate (Claus, 1988). Bacterial cultures were streaked on the medium and incubated at 30°C for 48 h. A clearing zone in the medium indicated positive enzyme activity.

2.3.7. Phosphate solubilization

Sterile Pikovskaya's agar plates supplemented with tricalcium phosphate were streaked with the endophytic bacterial isolates and incubated at 37°C for 10 days. Clear zone of phosphate solubilization around the growth was analysed (Tariq et al., 2014).

2.4. Abiotic stress tolerance assay

2.4.1. Salt tolerance estimation

Fresh bacterial culture was streaked on petri plates containing nutrient agar medium with different concentrations of sodium chloride (NaCl) viz 2%, 4%, 6% and 8%. Growth was observed after 24 h of incubation at 37°C (Ullah et al., 2018).

2.4.2. Antibiotic assay

Tetracycline, streptomycin sulphate, chloramphenicol, amoxicillin and bacitracin at 100 ppm, 150 ppm, 200 ppm and 250 ppm concentration were used to check the antibiotic sensitivity by following procedure of Kamble et al. (2017) with minor modification.

2.4.3. Antagonist activity of bacterial isolates in vitro

Antifungal activity against *Colletotrichum gloeosporioides* was tested on a sterile potato dextrose agar (HiMedia Laboratories Pvt. Ltd. India) plate, a disc of fungal pathogen was placed in the center and each bacterial isolate was inoculated at a distance of 2.5 cm from the fungal disc. The control plate was inoculated only with phytopathogenic fungi. The inhibition of mycelial growth was observed after incubation at 28°C for 7 days.

2.4.4. Compatibility against fungicide

Effective isolates which showed antagonistic activity were evaluated for compatibility by Kirby-Bauer test against all the registered fungicides mentioned in Annexure 5 (Anonymous, 2022), obtained from the Plant Pathology laboratory of ICAR-NRCG, Pune. Compatibility was studied according to Berger et al. (2009) with slight modifications (Table 1).

Table 1: Range of compatibility based on radial growth of inhibition zones (in mm)

Inhibition zone (mm)	Nature of compatibility
0–10.00	Highly compatible
10.1–20.00	Moderately compatible
20.1–50.00	Slightly incompatible
50.00 and above	Non-compatible

3. RESULTS AND DISCUSSION

3.1. Isolation of endophytes

Leaf samples from different varieties of grape were collected and subjected for surface sterilisation. Various types of bacterial colonies were grown on Nutrient Agar plates. Out of all, eighteen bacterial isolates were selected and purified on the basis of uniqueness in their colony morphology.

Ten isolates from white variety and 8 from coloured grape variety were selected. The isolates were named as MN1, NP2, TS3, TS13, TS14, CS4, MC5, MC8, MC9, MC10, MC24, MC30, MS6, FS7, 2A8, MSH1, MSH5 and S22.

3.2. Morphological characterization of the isolated endophytes

It was observed that out of 18 isolates, 5 were Gram negative and remaining were Gram positive but irrespective of Gram reactions all were rod shaped structures. It was observed that 5 isolates (TS14, MC10, MC24, MC30 and MSH5) which were Gram negative showed identical response in KOH test. MC 5, MC 30, 2A8 and MSH5 isolates had circular colony margin whereas all others had irregular margins. Colony size of the isolates varied from small to large and distinct pigmentation was observed in different isolates (Table 2).

Table 2: Growth of endophytic bacteria isolated from different grapes plant on nutrient agar media and their morphological characteristics with gram staining

Isolate name	Margin/ Edge	Colony size	Pigmentation	Gram's reaction	Shape
MN 1	I	IM	W	+	SR
NP 2	I	IM	WY	+	LR
TS 3	I	L	WY	+	LR
TS 13	I	S	W	+	SR
TS 14	I	S	WY	-	SR
CS 4	I	S	WR	+	SR
MC 5	C	S	W	+	SR
MC 8	I	S	WY	+	SR
MC 9	I	S	W	+	LR
MC 10	I	S	W	-	SR
MC 24	I	S	Y	-	SR
MC 30	C	S	Y	-	SR
MS 6	I	IM	W	+	SR
FS 7	I	IM	W	+	LR
2A 8	C	S	W	+	SR
MSH 1	I	L	W	+	SR
MSH 5	C	S	Y	-	SR
SS 22	I	L	W	+	SR

Keywords: I: Irregular, C: Circular, IM: Intermediate, S: Small, L: large; W: White; WY: Whitish yellow; WR: Whitish red; Y: Yellow; "+": Gram positive; "-": Gram negative; LR: Long rod; SR: Short rod

3.3. Biochemical characterization of endophytic bacterial isolates

3.3.1. Qualitative analysis of enzyme production

All isolates were catalase positive except TS14, MC10, MC24 and MC30. Isolates TS13, TS14, CS4, MC5, MC9, MC24, MC30, MSH1, MSH2 and SS22 were capable of producing urease enzyme. Six isolates viz. NP2, TS3, MC8, MC30, MS6 and 2A8 had produced gelatinase enzyme.

Results exhibited that 11 isolates viz. TS13, TS14, CS4, MC5, MC8, MC9, MC10, MC24, MC30, MS8 and MSH1 fermented dextrose sugar whereas 11 isolates (MN1, TS3, TS13, TS14, MC5, MC8, MS6, FS7, 2A8, MSH1, and MSH5) utilized glucose as sole source of carbon. Only TS13, TS14, MC24, 2A8, MSH5 and SS22 were using fructose sugar while TS13, MC24, MC30, MSH5 and SS22 were fermenting sucrose indicating that very few bacteria were using sucrose as sole carbon source (Table 4).

3.3.2. Screening of bacterial isolates for plant growth-promoting (PGP) traits and extracellular hydrolytic enzymes production

Out of 18 isolates, 11 bacteria (MN1, NP2, TS3, TS13, CS4, MC8, FS7, 2A8, MSH1, MSH2 and SS22) tested positive for amylase production (Table 3). All isolates except MC24, FS7, MSH2 and SS22 produced protease enzyme. Lipid was hydrolysed effectively by MN1, NP2, TS3, CS4, MC5, MC8, MC9, MC10, MS6, FS7 and 2A8.

All the isolates however, gave positive result for oxidase test. None of the endophytic bacteria showed hydrogen cyanide (HCN) production and phosphate solubilization. All isolated endophytes revealed positive result in ammonia production test except MC24, MSH 2 and SS22. Out of eighteen bacterial isolates, 10 isolates were positive for indole production (Table 3).

3.3.3. Antibiotic assay and salt tolerance test

All bacterial isolates showed zone of inhibition against tetracycline except isolate SS22. Zone of inhibition was not detected against tetracycline in SS22 bacteria at 100 ppm and 150 ppm concentrations. All bacteria were sensitive to streptomycin sulphate at all the tested concentrations. MC9, MC10, MC30, MSH1 and MSH5 showed resistance towards chloramphenicol at different concentrations. Zone of inhibition was not observed at any concentration of amoxicillin which manifested that all bacteria were resistant towards amoxicillin. Similar results were observed in bacitracin antibiotic except NP2, TS3, CS4, MC5 and FS7 (Table 5).

In salt tolerance test 4 different concentrations were tested and it was observed that all isolates except CS4, MC8, MC9 and MSH1tolerated higher i.e. 8% NaCl concentration. MC24 and MC8 had salt tolerance upto 2% and 4% respectively (Table 4).

3.4. Antagonistic activity

The biocontrol activity of the eighteen selected strains were evaluated *in vitro* against *Colletotrichum gloeosporioides*. Ten strains (TH 13, TH 14, MC8, MC10, TH3, CS4, FS7, MN1, 2A8 and MC5) effectively inhibited *Colletotrichum*

Table 3: Biochemical assay of endophytic bacteria; Keyword: "+"=positive, "-"=negative

Isolate name	Amylase test	Catalase test	Urease test	Lipase test	Protease test	Gelatinase test	HCP	Ammonia production	Phosphate solubilization	PHT	Indole test
MN 1	+	-	-	+	+	-	-	+	-	-	-
NP 2	+	-	-	+	+	+	-	+	-	-	-
TS 3	+	-	-	+	+	+	-	+	-	-	-
TS 13	+	+	+	-	+	-	-	+	-	-	+
TS 14	-	-	+	-	+	-	-	+	-	+	+
CS 4	+	-	+	+	+	-	-	+	-	-	-
MC 5	-	-	+	+	+	-	-	+	-	-	-
MC 8	+	+	-	+	+	+	-	+	-	-	+
MC 9	-	+	+	+	+	-	-	+	-	-	+
MC 10	-	-	-	+	+	-	-	+	-	+	+
MC 24	-	-	+	-	-	-	-	-	-	+	+
MC 30	-	-	+	-	+	+	-	+	-	+	+
MS 6	-	-	-	+	+	+	-	+	-	-	-
FS 7	+	-	-	+	-	-	-	+	-	-	-
2A 8	+	-	-	+	+	+	-	+	-	-	-
MSH 1	+	+	+	-	+	-	-	+	-	-	+
MSH 5	+	+	+	-	-	-	-	-	-	+	+
SS 22	+	+	+	-	-	-	-	-	-	-	+

HCP: Hydrogen cyanide production; PHT: Potassium hydroxide test

gloeosporioides growth. Maximum inhibition was observed by MC8, MN1 and MC10 followed by CS4. Other six isolates showed intermediate results, whereas rest all isolates were unable to inhibit the fungus showing full growth on the Potato Dextrose Agar plates. On the basis of antifungal property of all isolates, ten strains viz. TH 13, TH 14, MC8, MC10, TH3, CS4, FS7, MN1, 2A8 and MC5 were selected for compatibility studies.

3.5. Compatibility against fungicides

Compatibility of endophytes isolated from grapevine against registered fungicides was studied. The concentration of used fungicides was in accordance with Annexure V established by ICAR-National Research Centre for Grapes, Pune (Anonymous, 2021).

It was observed that all isolates were highly compatible with 8 fungicides registered for downy mildew viz. fosetyl Al 80 WP, ametoctradin 27+dimethomorph 20.27 SC, dimethomorph 50 WP, azoxystrobin 23 SC, kresoxim methyl 44.3 SC, fluopicolide 44.44%+fostyl Al 66.67% WG, dimethomorph 12%+pyraclostrobin 6.7% WG and cyazofamid 34.5% SC. All bacterial isolates demonstrated high compatibility with 9 registered fungicides for powdery mildew including triazole groups viz. tebuconazole, difenoconazole, penconazole, hexaconazole, and flusilazole, sulphur based fungicides, strobilurin fungicides like azoxystrobin, trifloxystrobin,pyraclostrobin. However, only meptyldinocap 35.7% EC, was incompatible to all isolates. It was observed that all the isolates were highly compatible with fluopyram 200+tebuconazole 200SC, carbendazim 50 WP, 46.27 SC and thiophanate methyl 70 WP registered for anthracnose disease as well as with kasugamycin 5%+copper oxychloride 45% WP fungicide registered for bacterial leaf spot. However, dithiocarbamates such as mancozeb, propineb along with their combinations had deleterious effect on all the bacteria.

3.6. Discussion

Endophytes facilitate plant growth by protecting plants from plant pathogens and increasing their tolerance against various biotic and abiotic stresses (Khanna et al., 2019). In the present study, 18 morphologically distinct endophytic bacteria were isolated from the leaves of seven coloured and three white varieties of grapes. Endophytes have already been reported successfully as biocontrol agents (BCAs) against several phytopathogens (Fadiji et al., 2020). West et al. (2010) stated that grapevines potentially contain a diverse array of bacterial endophytes, including species common to

Table 4: Sugar utilization test and salt tolerance test Keyword: "+"=positive, "-"=negative

Isolate name	Sugar utilization test (1%)			Salt tolerance test			
	Dextrose	Glucose	Fructose	Sodium chloride (2%)	Sodium chloride (4%)	Sodium chloride (6%)	Sodium chloride (8%)
MN 1	-	+	-	+	+	+	+
NP 2	-	-	-	+	+	+	+
TS 3	-	+	-	+	+	+	+
TS 13	+	+	+	+	+	+	+
TS 14	+	+	+	+	+	+	+
CS 4	+	-	-	+	+	+	-
MC 5	+	+	-	+	+	+	+
MC 8	+	+	-	+	+	-	-
MC 9	+	-	-	+	+	+	-
MC 10	+	-	-	+	+	+	+
MC 24	+	-	+	+	-	-	-
MC 30	+	-	-	+	+	+	+
MS 6	+	+	-	+	+	+	+
FS 7	-	+	-	+	+	+	+
2A 8	-	+	+	+	+	+	+
MSH 1	+	+	-	+	+	+	-
MSH 5	-	+	+	+	+	+	+
SS 22	-	-	+	+	+	+	+

the vines immediate environment.

According to the morphological and physiological analyses, the majority of bacterial isolates were Gram positive. Previous reports showed that Gram-positive bacteria (61.73%) were isolated more frequently than Gram-negative bacteria from grapevine plant tissues (Altalhi, 2009) while they were reported to be of equal proportionate in grapes (Zinniel et al., 2002). In contrary, earlier workers have even reported a predominance of Gram negative bacteria in the tissues of various plants.

In the present study morphological characterization of indigenous isolates of grapevine showed that the colonies varied from circular to irregular shaped while the margin varied from entire to irregular. Colony colour was diverse from white to yellow. A similar pattern of results in terms of cell shape, colour and margins were observed in other studies as well (Sgroy et al., 2009, Kumar et al., 2015).

Bacteriological approaches like morphological and biochemical characterization for the identification of endophytic bacteria had been used in many investigations (Silva and Nahas, 2002). Ten isolates were positive for indole production which were in disagreement with the findings of Bhagya et al. (2019) who had isolated endophytic bacteria from nodule, root and seeds of greengram (*Vigna radiata* L.) but they were indole negative. However, experiments conducted by Kumari et al. (2021) showed indole positive results by the endophytic *Bacillus* isolates. Current study revealed that 11 isolates had utilized dextrose and glucose as sole source of carbon which were in agreement with the previous findings by Pal et al. (2012), who reported that out of 20 endophytic bacteria isolated from *Paederia foetida* L. 14, 12, and 5 isolates had utilised dextrose, fructose and sucrose respectively as carbon source.

Endophytes have to tolerate different environmental factors like fluctuation in temperature and salinity. In the present finding, all the isolates tolerated up to 6% salt concentration except MC8. Thirteen isolates were found to be salt tolerant up to 8%. Several other investigations had also reported that endophytic bacteria efficiently tolerated the high salt concentration (Mohamad et al., 2020). Jasmin et al. (2014) isolated 15 endophytic bacteria from ginger (*Zingiber officinale Rosc.*), and out of which 6 and 9 tolerated up to 7–8% of NaCl and10% NaCl respectively.

The study revealed that all isolates were able to produce amylase, lipase and protease at varying levels. These enzymes including amylase, lipase, protease etc. participate in the

Table 5: Estimation of zone of inhibition of different antibiotics at different concentration; Keyword: "+"=positive, "-"=negative

Isolate	Tetracycline (ppm)				Streptomycin sulphate (ppm)				Chloramphenicol (ppm)				Amoxicillin (ppm)				Bacitracin (ppm)			
	100	150	200	250	100	150	200	250	100	150	200	250	100	150	200	250	100	150	200	250
MN 1	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	+
NP 2	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+
TS 3	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+
TS 13	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
TS 14	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
CS 4	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+
MC 5	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+
MC 8	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
MC 9	+	+	+	+	+	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-
MC 10	+	+	+	+	+	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-
MC 24	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
MC 30	+	+	+	+	+	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-
MS 6	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
FS 7	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+
2A 8	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	+
MSH 1	+	+	+	+	+	+	+	+	-	-	-	+	-	-	-	-	-	-	-	-
MSH 5	+	+	+	+	+	+	+	+	-	-	+	+	-	-	-	-	-	-	+	+
SS 22	-	-	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-

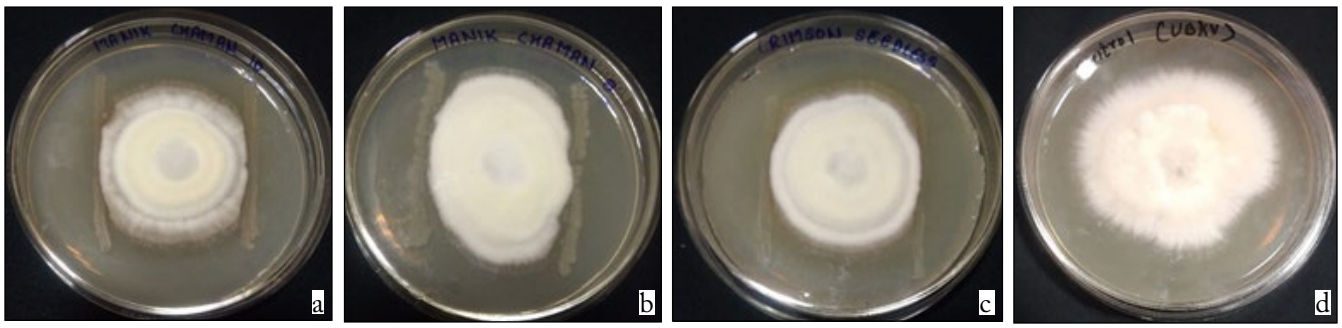

Figure 1: Antagonistic activity of endophytes (a- MC10, b-MC8, c-CS4, d- control) against pathogen *Colletotrichum gloeosporioides*

antagonistic activity by disrupting cell wall of the pathogen (Ross et al., 2000). Antagonistic activity of endophytic bacteria mostly depended on the secretion of different hydrolytic enzymes (Compant et al., 2005). Yasmin et al. (2010) demonstrated that when isolates produced clear zones, they are phosphate solubilizers but the studied isolates did not exhibit any phosphate solubilising capacity.

Ammonia and hydrogen cyanide production had the ability to suppress pathogen development and indirectly stimulated plant growth. Contradictory results were observed in the study, where no isolates had shown positive results for HCN and ammonia production. Even though the results were not in favour of antagonism by HCN and ammonia production, 10 isolates had significantly inhibited the growth of pathogenic fungus *Colletotrichum gloeosporioides.* The results suggested that endophytes may have produced the hydrolytic enzymes or exhibited different mechanism to inhibit the fungus. Many of the previous studies have shown that endophytic bacteria controlled fungal pathogens as well as *Bacillus* sp. (Kumar et al., 2016, Mohamad et al., 2020). Antagonistic effects of bacterial endophytes on various pathogens of agriculturally important crops had been demonstrated earlier (Berdy et al., 2005, 2002, Costa et al., 2013).

Figure 2: Compatibility of endophytes against various fungicides; a- MC24 with hexaconazole (highly compatible), b-2A8 with Copper Sulphate 47.15%+Mancozeb 30% WDG (non-compatible), c-MC24 with mancozeb (non- compatible)

All the bacterial endophytes were susceptible to tetracycline, streptomycin sulphate and chloramphenicol while resistant to other antibiotics used in the study. The findings were not in line with previous findings of Kumar et al. (2016) who suggested that bacterial endophyte isolate CT5 of *Cassia tora* L. was resistant to chloramphenicol. But in his report, it was also revealed that the isolate was resistant to amoxicillin which favoured the present findings. Such contrasting results indicated that the behaviour of bacterial endophytes may vary from plant to plant and from species to species depending on the environmental conditions (Nair and Padmavathy, 2014). The antibiotic properties of endophytic bacteria increased the host plant resistance to pathogens and promoted their growth (Bhore et al., 2010).

The compatibility study of endophytes was conducted against all fungicides of downy mildew,powdery mildew, anthracnose and bacterial leaf blight by disk diffusion technique. Depending on the antifungal activity, 10 endophytes (TH 13, TH 14, MC8, MC10, TH3, CS4, FS7, MN1, 2A8 and MC5) were taken into account for the compatibility study. Our findings on compatibility study hinted that all the endophytes can be used against fungal diseases with various fungicides. Understanding the compatibility between endophytes and fungicides provided a wider perspective on the use of integrative methods in disease management (Lima et al., 2006). Daniel et al. (2022) had also reported that *Bacillus subtilis* was compatible with five fungicides viz. Carbendazim 50% WP, Hexaconazole 5% SC, Thiophanate Methyl 70% WP, Azoxystrobin 18.2%+Difenconazole 11.4% SC and Azoxystrobin 23% SC. The combination of antagonistic endophytes and fungicides might also influence biocontrol activity by metabolizing antibiotic substances and inhibiting the growth and development of plant pathogenic fungi (Thahir et al., 2010). Management of fungal diseases such as downy mildew and powdery mildew in grapes is indeed challenging task. It was also observed that using endophytes in combination with fungicides can reduce the usage of fungicides on the crop. The reason for compatibility between the fungicides and endophytic isolates cannot be narrowed down, it may be so as the isolates might already be immune towards the fungicides or might be using the same as a source of nutrition. Since fungicides are less potent towards bacteria, beneficial bacteria can slowly adapt to the environment and colonize the plant better (Ons et al., 2020; Vyas and Kaur, 2021).

4. CONCLUSION

This study demonstrated that grapevine harbors highly diverse endophytic bacteria. Eighteen endophytes from nine different varieties of grapevines were isolated. Biochemical characterization, antagonistic activity against fungus *Colletotrichum gloeosporioides* and compatibility study with the fungicides were performed, which manifested that some isolates might be good candidates for use as biocontrol agents. Further molecular characterization and their effects on plant growth under pot and field conditions would help us to understand the plant microbe interaction in details.

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