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Mechanism of Action of Effective Microbes Against *Fusarium oxysporum* f. sp. *melonis in vitro*

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Abstract The use of effective microbes obtained from the rhizosphere to suppress soil borne plant pathogens has received greater attention in recent decades as an alternative to chemical fungicides. The present study was conducted to explore the effects of effective microbes (EMs) and to understand the mechanisms of action and their ability to produce inhibitory metabolites and hydrolytic enzymes that enhance their antagonistic capability and activity against Fusarium wilt caused by *Fusarium oxysporum* f. sp. *melonis* (Fom), as well as plant growth promotion activity on the rock melon plant. Seven EMs namely *Bacillus amyloliquefaciens* MKB04 (KM220772). MKB09, *Alcaligenes faecalis* MKB10 (KM220771), MKB12, MKB15, MKB24 and MKB37 were selected, The *in vitro* evaluation of antifungal compound production was found to be a common characteristic among the seven selected potential bacterial isolates. Moreover, the isolates showed strong antagonistic activity and different mechanisms of action in the diffusible metabolites assay compared to the volatile one, This characteristic significantly add to its role in controlling Fom, where the bacterial isolates exhibited significantly different combinations of antimicrobial metabolites, such as cellulase, pectinase, proteases and chitinases. The activities of the various effective bacteria isolates were shown by the clear zones that formed surrounding the colonies of bacteria. Furthermore, the isolates responded positively *in vitro* for siderophore and HCN, indole acetic acid (IAA) production, and phosphate solubilisation. As effective microbes are environmental friendly and in vitro antagonistic and hydrolytic activities it showed against *F.oxysporium f. s. melonis* in this study suggest that they can be used as an effective biological control agent as well as plant growth promoters.

Keywords: *Biological control, Effective microbes (EM), Fusarium wilt, Growth promoters*

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1. Introduction

Beneficial bacteria genera are found in the soil rhizosphere, on the surfaces of roots and associated with the plant roots which can enhance plant growth directly or indirectly [1,2,3]. The direct stimulation by beneficial bacteria involve either offering the plant with plant growth promoting substances that are produced by the bacterium or assisting the uptake of certain nutrients from the environment. The indirect promotion of plant growth happens when the beneficial bacteria decrease or inhibit the detrimental effect of one or more phytopathogens [4].

Many rhizobacteria, Beneficial bacteria genera stimulate plant growth via various mechanisms. They affect germination rate, as well as crop yield under adverse conditions. Because of the different metabolites they produce, the use of microbes in agriculture as Biocontrol agents against plant pathogens and pests, as well as for biological disease control, could offer a viable option for plant disease control [5,6,7].

The exact mechanisms by which these bacteria operate have still to be fully appreciated, in the direct mechanism, rhizobacteria stimulate crop production by enhancing the soil nutrients for e.g., a symbiotic nitrogen fixation [8,9], solubilisation of mineral phosphate potassium, and iron and other nutrients [10,11], Indirect mechanisms involve plant growth promotion by producing antagonistic substances such as hydrogen cyanide (HCN) production [12], production of enzymes, siderophores [13], different phytohormones [14] and antagonism against phyto-pathogenic microorganisms and biofilm formation [15,16,17,18]. Understanding the mechanisms and types of actions is essential for their utilization in agriculture to promote sustainable and eco-friendly system. They play crucial roles in enhancing plant health, nutrient uptake and overall crop productivity.

The present study was carried out to understand the mechanisms of action, the ability of seven effective isolates namely MKB04, MKB09, MKB10, MKB12, MKB15, MKB24 and MKB37, [19] to produce inhibitory metabolites and hydrolytic enzymes that enhance their antagonistic capability and activity, and their impact on the cell integrity of the pathogenic fungus *Fusarium oxysporum* f.sp.*melonis* as well as plant growth promotion activity.

2. Materials and Methods

2.1. Production of Inhibitory Compounds

2.1.1. Production of Non-volatile Antifungal Compounds

A non-volatile compound(s) was produced in bi-layer agar that was modified from the cellophane method, as reported by [20] As the basal medium 15 ml PDA was poured into 9 cm diameter sterile Petri dishes and allowed to solidify. A nine cm sterilized Whatman® filter paper was placed aseptically on the solid medium, and 10 ml NA medium was then poured over it. Effective bacteria of 48-hour culture were streaked onto the surface of the media. The plates were incubated at 28±2°C for three days and filter papers with NA were removed carefully; then the Centre of the PDA plates was inoculated with a six mm mycelial plug of five-day-old culture of Fom. Co-inoculation was incubated for an extra seven days and the radial growth of the Fom was recorded. Control treatments were run with the Fom culture on the non-treated PDA plates.

2.1.2. Production of Volatile Antifungal Compounds

The ability of effective bacterial isolates to produce volatile compound(s) was also evaluated in this study. A double plate test was prepared consisting of two different media. Effective bacteria of 48-hour culture were streaked onto the surface of the NA plate media, and a six mm mycelial plug of five-day old culture of Fom was placed at the centre of the PDA plates. The lids of both plates containing the effective bacteria and the pathogen were removed. Fom agar plates were inverted over the effective bacteria culture plates and sealed with Para film to prevent loss of the volatiles produced. The plates were then incubated at 28±2°C for seven days. The growth of Fom was measured and compared to the control plate [21]. The results were assessed as the percentage inhibition of the pathogen growth in the presence or absence of effective bacterial isolates.

2.2. Production of Hydrolytic Enzymes

2.2.1. Plate Assays for Hydrolytic (cellulolytic, pectinolytic, proteolytic and chitinolytic) activities

The effective bacterial isolates MKB04, MKB09, MKB10, MKB12, MKB15, MKB24 and MKB37 were cultured in Petri plates for 24hr at 30°C in NA medium. One full loop of each effective bacterial was spotted onto plates which contained the substrate of the enzyme to be tested and grown at 25°C for48hr.

Cellulolytic activity was measured according to [22] employing a solid medium, that contained: 0.1g $MgSO_4 7H_2O$, 0.2g $CaCl_2 2H_2O$, 0.04g $FeSO_4 7H_2O$, 0.2g NaCl, 0.3g KH_2PO_4, 0.5g K_2HPO_4, 5g CMC (carboxymethylcellulose) (Sigma), 0.1g yeast extract, 15g Bacto agar and 1L Water. The hydrolysis was visualised by flooding the agar medium that contained CMC with Congo red solution (1mg/ml) for 15min.

Pectinolytic activity was assessed as indicated by [23] with slight modification using normal pectin. The plate medium consisted of 2.0g $(NH_4)_2SO_4$,6gNa_2HPO_4,1mg$FeSO_4 7H_2O$, 4g KH_2PO_4, 10µg H_3BO_3, 50µg $CuSO_4$,0.2g $MgSO_4$, 10µg $MnSO_4$, 10µg MoO_3, 1mg $CaCl_2$,70µg $ZnSO_4$, 5g pectin (Sigma), 1g yeast extract, 15g Bacto agar 1L water. Pectinolytic activity was observed by flooding plates for 10 min with a 1% solution of hexadecyl trimethyl ammonium bromide (HDTAB) in water. Enzyme activities were determined when a clear zone (halo) developed around the colonies of bacteria.

Protease activity was shown in the form of casein degradation, with bacterial isolates plated on agar medium that contained 100g skimmed milk (Difco), 1.5g yeast extract and 15g Bacto agar, and observed directly on the plates after 48hrand the width of each resulting clearing halo was recorded as an indicator of the level of protease activity [24,25].

Chitinolytic activity was determined according to the method of [26]. The bacterial isolates chitinase activity on chitin-agar (CA) plates. Chitin powder was used in the preparation of colloidal chitin by modifying the technique of [27]. The chitin powder was slowly added to 10N HCl and stored overnight at 4°C with vigorous stirring. The suspension was added to 50% cold ethanol with vigorous stirring and stored overnight at 25°C. The precipitate was gathered by centrifugation at 10000 rpm for 20 min and rinsed with sterile distilled water until the colloidal chitin was neutral (pH 7.0). The chitin-agar (CA) contained 1 g $NH_4H_2PO_4$, 0.2 g KCl, 0.2 g $MgSO_4 7H_2O$, 1% (w/v) colloid chitin, 20 g agar, 1000 ml distilled water, pH 7.0. The chitin degradation by the two isolates was evaluated by spot inoculating on CCA and incubated at room temperature. The zone of clearance due to chitin hydrolysis was observed after five days.

2.3. Plant Growth Promotion Activities

2.3.1. Phosphate Solubilisation

The ability of effective bacteria isolates to solubilize phosphate was assessed on modified Pikovskaya agar plates with insoluble tricalcium phosphate (TCP)(National Botanical Research Institute's phosphate growth medium (NBRIP), 10 g Glucose,5g $Ca_3(PO_4)_2$,5g $MgCl_2.6H_2O$, 0.25g $MgSO_4.7H_2O$, 0.2g KCl,0.1g $(NH_4)_2SO_4$, 1.5% Bacto agar (Difco USA),Water 1L.A full loop of each culture was placed on the centre of the agar plates and incubated at 30±0.1°C for 3 days. The un-inoculated control was also kept under similar conditions. The solubilisation clear zone was determined by subtracting the diameter of the bacterial colony from the diameter of the total zone.

2.3.2. Production of Indole-3-Acetic Acid (IAA)

Fifty milliliters of nutrient broth (NB) with 0.1% DL tryptophan was inoculated with 500 µl of 24-hr-old effective bacterial cultures and incubated in a shaker at 30±2°C and 150 rpm for 72 hr. The bacterial cultures were centrifuged at 10,000 rpm for 10 min. Detection of indole-3-acetic acid (IAA) in the supernatants was achieved employing colorimetric assay [28].

The concentration of IAA produced by the effective

bacteria was tested by mixing one milliliter of supernatant from each isolate with 2 ml Salkowsky reagent (150 ml of concentrated H_2SO_4, 250 ml of distilled H_2O, 7.5 ml (0.5) M $Fe_3Cl_2.6H_2O$ [29]; one milliliter of NB without culture was mixed with the Salkowsky reagent as control. The mixtures were incubated for 20-25 min at room temperature; and the absorbance of the resultant pink colour readings was taken at 530 nm using a UV/Visible Spectrophotometer [28]. Pure IAA was used for preparing the standard curve and the IAA production was calculated from the regression equation of the standard curve and the result was expressed as µg ml $^{-1}$ over control.

2.3.3. Ammonia Production

Ammonia production by bacterial isolates was tested as described by [30]. Bacterial cultures of 24 hrs old were inoculated in 10 ml peptone broth and incubated at 30±2°C for 48 hrs in a shaker. Following incubation 0.5 ml of Nessler's reagent was added. The change of the colour to light yellow or dark brown indicated the ammonia production.

2.3.4. Hydrogen Cyanide Production

The hydrogen cyanide (HCN) production of effective bacterial isolates was tested, as described by [31]. Bacterial cultures were streaked on nutrient agar medium containing 4.4 g per litre of glycine. A Whatman filter paper No. 1 was soaked in 0.5% picric acid solution in 1% sodium carbonate and then placed in the upper lids of the plates. The plates were sealed with Para film and incubated at 30±1°C for 4 days. The turn of filter paper colour to light brown or dark brown colour indicated HCN production.

2.3.5. Siderophore Activity

For siderophore production, an iron free modified Succinate medium (SM) of [32]. Consisting of (g/L): 6.0 K_2HPO_4, 3.0 KH_2PO_4, 0.2 $MgSO_47H_2O$, 1.0 $(NH4)_2SO_4$, 4.0 succinic acid, pH 7.0 with slight modification by adding 15g agar was used. CAS agar plates were prepared as CAS (60.5 mg) was dissolved in distilled water (50 mL) and blended with 10 ml of iron (III) solution (1 mM $FeCI_3.6H_2O$ in 10 mM HCI) with stirring. This solution was gradually added to 72.9mg of HDTMA dissolved in 40 ml of water. The dark blue liquid that resulted was autoclaved at 15 lb psi and then mix with the modified SM. Siderophore activity was determined on Chrome-Azurol S (CAS) medium according to the procedure of [33]. The effective bacterial isolates of 24-hrold cultures were each spotted on the CAS medium and incubated at 25±2 C for 48–72hr ([13,34]). Formation of an orange to yellow halo against a dark blue background around the colonies confirmed the siderophore production.

2.4. Statistical Analysis

The experiments were conducted in Completely Randomized Design (CRD) with five replicates. Recorded data were analyzed with SAS® Software. Statistical significant data were determined using Duncan's Multiple Range Test ($p<0.05$). The percentage data were transformed into Arcsine transformation before subjected to ANOVA [35].

3. Results

3.1. Mechanism of Action of Effective Microbes against Fom

3.1.1. Production of Inhibitory Compounds

The seven isolates were found to produce diffusible as well as volatile metabolites, with evidence of considerable differences with the control ($p < 0.05$) in terms of mycelial growth inhibition. All the bacterial isolates tested have a good ability to inhibit the mycelial growth of Fom by producing diffusible antifungal compounds with PIRG values 66.4% to 14.8% and 90% to 65% by volatile metabolites and non- volatile metabolites for the seven isolates, respectively (Table 1). On the other hand, the mycelial growth of Fom on PDA was inhibited by volatile metabolites produced by the bacterial isolates after 7 days of incubation, where the isolate MKB04 showed the higher inhibitory effect 54.63%, compared to the rest isolates, which showed an inhibition percentage of < 50% with respect to the control as shown (Table1).

Table 1. Percentage of Effective bacteria with potential against Fom based on their respective PIRG values

Isolate	Volatile Metabolites%	Nonvolatile Metabolites%
MKB04	66.5^{a}(54.63)	88.07^{b}(69.79)
MKB09	53.4^{b}(46.95)	85.8^{d}(67.86)
MKB10	49.4^{c}(44.66)	86.36^{c}(68.33)
MKB12	25^{f}(30.00)	84.09^{e}(66.49)
MKB15	34.1^{d}(35.73)	81.82^{f}(64.76)
MKB24	14.8^{g}(22.63)	65.91^{g}(54.25)
MKB37	31.8^{e}(34.33)	90.34^{a} (71.80)
control	0	0

Data are means of five replicates. Means in a column followed by different letter are significantly different according To DMRT at $P \leq 0.05$. (% Data are arcsine transformed)

3.1.2. Production of Hydrolytic Enzymes

The plate assays for the hydrolytic enzyme (Proteolytic, cellulolytic, pectinolytic and chitinolytic) activities of the varied beneficial bacteria isolated from the rhizosphere of rock melon are shown in Table 2. The hydrolytic activities of the two isolates, as shown by the clear zones formed surrounding the bacterial colony, were identified in the medium supplemented with skimmed milk (Plate 1), CMC, and pectin, with diameters of lytic zones of 3- 43, 2- 39 and 9 - 22 mm, respectively (after 48h incubation at 28°C).

In addition, the seven studied isolates were positive for chitinolytic activity five days after incubation at 28°C. the formation of clear zones were observed for six isolates two of which show weak chitinlytic activity namely MKB09 and MKB37. No chitinlytic activity was observed for MKB24. The production of the fungal cell wall degrading enzymes was analysed as this is a crucial mechanism related to the inhibition of fungus.

Table 2. Cellulolytic, Proteolytic, Chitinolytic and Pectinolytic activity of the seven effective bacteria

Bacteria isolates	Hydrolytic activity			
	Cellulase	Protease	Pectinase	Chitinase
MKB04	+	+	+	+
MKB10	+	+	+	+
MKB04	+	+	+	+
MKB09	+	+	+	+
MKB10	+	+	+	+
MKB12	+	+	+	+
MKB15	+	+	+	+
MKB24	+	+	+	−
MKB37	+	+	+	+

Presence (+) or absence (−)

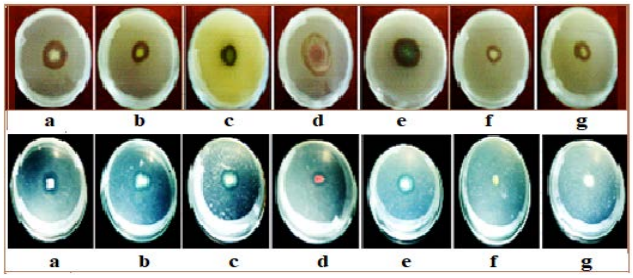

Plate 1. Proteolytic (Upper) and chitinolytic enzymes activity (lower) by a: MKB04; b: MKB09; c: MKB10; d: MKB12; e: MKB15; f: MKB24; MKB37 against Fom on PDA (after 3 days' incubation).

3.1.3. Growth Promotional Activity

Growth promotional activity of the selected bacteria and their activity against the Fusarium wilt of rock melon pathogen In-vitro activity of EMs for P solubilization as indicated by the formation of halo zones surrounding the bacterial colony, Cyanide hydrogen, siderophores and IAA by brown to brick, yellow and pink colouration respectively were detected in medium supplemented with specific reagents (after 5 days incubation at 28°C) as shown in Table 3.

Test on the ability of effective bacterial isolates to solubilise phosphate showed that all isolates gave clear zone on phosphate plates after incubation for 5 days. There is a significance difference ($P \leq 0.05$) in the clear zones produced by bacterial isolates. The result indicates that the effective bacterial isolates that produced clear zones on phosphate agar are able to solubilize phosphate.

All the seven tested effective bacteria isolates namely MKB04, MKB09, MKB10, MKB12, MKB15, MKB24 and MKB37 responded positively to production of phytohormone, IAA by the production of pinkish colour, where isolates exhibited the highest intensity (+++) of pink colour, indicates higher production capacity for IAA (Table 3). All isolates gave significance levels of IAA ranging from 44.25µg/ml to 59.56µg/ml, with significant difference between seven isolates.

All isolates have ability to produce siderophores as evidenced formation of orange halo around the colony due to the release of iron from the iron-dye complex indicating the production of a siderophore (plate 2), MKB04 isolate showed bigger halo than other isolates.

Production of HCN was indicated by change in colour of the filter paper to brown. Only three effective isolates showed strongly colour change, MKB09, MKB15 and MKB37. The other four isolate showed slight colour change (Table 3). Moreover, development of yellow-brown colour was observed after addition of Nessler's reagent indicating a positive test for ammonia production. It has been reported that ammonia production indirectly influences the plant growth.

Different mechanisms of the effective isolates have been observed for their positive performance such as siderophore, HCN and indole acetic acid (IAA) and urease production,Phosphate solubilization, and nitrate reduction, the production ability of all bacterial isolates was significantly ($p<0.05$) varied.

Table 3. *In-vitro* Plant Growth Promotional Activities of Effective Bacterial Isolates

Isolate	Phosphate Solubilization (mm)	Hydrogen Cyanide	Siderophores	IAA
MKB04	+	+	+++	+++
MKB09	+	+	+	++
MKB10	+	+	++	++
MKB12	+	-	+	+++
MKB15	+	-	+	++
MKB24	+	-	+	-
MKB37	+	+	+	+++

Presence low(+), moderate (++), high(+++) or absence (−)

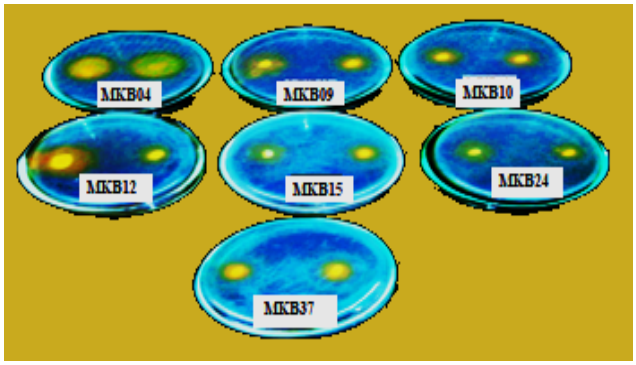

Plate 2. Siderophore produced by effective bacteria on CAS agar plate test (after 4 days incubation)

3.2. Discussion

The *in vitro* evaluation of antifungal compound production was found to be a common characteristic among the seven selected potential bacterial isolates. Moreover, the isolates showed strong antagonistic activity in the diffusible metabolites assay compared to the volatile one. This characteristic might significantly add to its role in controlling Fom; similar findings were previously demonstrated by [21]. The inhibition produced as shown by a clear zone in the *in vitro* experiment is evidence of antibiosis by the bio-control agent against the fungal pathogens, thus suggesting that the antibiotic substances produced by the bacterial isolates were fungistatic as well as fungicidal to the spores of Fom. These findings are supported by many researchers [36,37,38,39] who described the inhibition of mycelial growth, disruption of fungal cytoplasmic membrane inhibition of the spore germination of several fungi by *Bacillus*, *Burkholderia*,

Pseudomonas, and *Serratia* spp.

The results of the *in vitro* test showed that different isolates exhibited significantly different combinations of antimicrobial metabolites (volatile and non-volatile) that inhibited the mycelial growth and spore germination of Fom. Thus, these isolates can be considered as potential biological control agents to control the Fusarium wilt of rock melon.

Proteolytic, cellulolytic, pectinolytic and chitinolytic activities of the various effective bacteria isolated from the rhizosphere of rock melon were shown by the clear zones that formed surrounding the colonies of bacteria. Extracellular lytic enzyme production is typical of antagonistic microorganisms [40,41]. These enzymes act and are participate in the antagonistic activity in various ways; most of them can have an effect on the cell wall of pathogens, which has been recorded for cellulases, chitinases and proteases produced by many bacteria [42]. As,mentioned earlier, analysis was made of the production of fungal cell wall degrading enzymes as this is crucial mechanism in the inhibition of fungus. Chitinases, the lytic enzymes produced by several bacteria that specifically degrade chitin, have received more attention in research [43,44,45]. It has been isolated from many bacterial genera including *Bacillus* and *Pseudomonas* [46,47,48,49]. It was found in this study that the isolates secreted chitinase, which allows the bacteria to degrade the fungal cell-wall. Extracellular chitinase production is considered crucial for the antagonistic activity of bacterial isolates [50,51,52]. The production of chitinase could be involved in the biocontrol of Fom.

Generally the inhibition of pathogen growth and hypha malformation could be due to the production of hydrolytic enzymes. The results of the *in vitro* test of hydrolytic activities of effective bacterial isolates showed that different isolates exhibited significantly different combinations of antimicrobial metabolites, such as pectinase, proteases and chitinases.

Regarding the growth promotional activity of selected bacteria and their activity against the Fusarium wilt of rock melon pathogen, this study showed the ability of effective bacterial isolates to solubilise phosphate as all selected isolates gave a clear zone on the phosphate plates. This investigation was found to be similar to the result of other studies [53,54] that clearing zones produced by isolates were chosen to be phosphate solubilizers. These phosphate solubilizers could promote plant growth as they are able to solubilize phosphate into soluble form that can be easily taken by plants as different organic acids are released by these isolates, which decreases the pH of the culture media results in phosphate solubilization, Various studies revealed the recent trends, progress and development with respect to mineral phosphate solubilisation by various plant growth promoting rhizobacteria genera belonging to Bacillus, *Alcaligenes faecalis*, Peudomonas and Klebsiella as reported by [55,54,56,57] [58,59,60,61,62]

On the other hand, the seven effective bacteria isolates tested, (MKB04, MKB09, MKB10. MKB12, MKB15, MKB24 and MKB37) positively responded to the production of phytohormone IAA by the production of a pinkish colour. This result agreed with [63,64] who demonstrated that different bacterial species can produce IAA. Generally, indole acetic acid (IAA) is universally accepted as a plant growth promoter. It has been recorded that IAA production by bacteria improves the way the host plant root system develops and as such favours the growth of crop plants [28] Furthermore, microbial acid production is of importance in the suppression of plant pathogens [65,66,67] proved that IAA could reduce spore germination, mycelial dry weight and the protein content of the pathogenic fungi and thus, prevent significant disease induction by soil pathogens. These findings were complemented by demonstrating the significant production of IAA by all isolates in the different genera in the present study results. The results of the present study are in line with the studies of [58,68,69], who reported the production of IAA by various genera of bacteria belonging to Bacillus, Pseudomonas, *Alcaligenes faecalis*, Serratia and Klebsiella .It has been documented that the production of IAA by PGPR differs from species to species and from strain to strain and is also impacted by conditions of culture, stage of growth and the availability of substrate [64].

The role of iron chelating siderophores in plant growth promotion is well documented by [70]. All seven isolates have the ability to produce siderophores as evidenced by the formation of an orange halo around the colony due to the release of iron from the iron-dye complex indicating the production of a siderophore. The MKB04 isolate showed a bigger halo than the others. Colour changes from blue to orange were reported by [71]. Siderophores are low-molecular weight, iron-chelating ligands produced by microbes in order to combat Fe insolubility [72]. The effective isolates were characterized for the production of siderophores, hence, the ability to produce siderophores and chelate nutrients, which are essential for the proliferation of pathogens, is an indirect mechanism by which rhizoshere microorganisms promote plant growth. Soil bacterial isolates such as *Azotobacter*, *Pseudomonas* and *Bacillus* siderophores were found to significantly increase crop yield and provide competitive inhibition to the growth of soil-borne pathogens [73,74]. In addition, with microorganisms competing against fungal pathogens for the available iron, the role of siderophores can be considered as a direct mechanism of biocontrol for the organic compounds needed for reactivation of propagules and root colonisation by the soil-borne pathogen [75,76].

HCN is a volatile, secondary metabolite that suppresses the development of pathogen and that affects the growth and development of [77]. In terms of the production of HCN, the seven effective isolates showed a slight colour change into brown, which was observed in the filter paper test. HCN is a volatile, secondary metabolite that suppresses the development of pathogens and indirectly influences the growth and development of plants [77]. Isolates from the rhizosphere soil of many plants, and contaminated soil showed HCN production [78,79]. Microbial production of HCN has been documented as a significant antifungal to control fungi from infecting the root. Moreover, another volatile compound ammonia was produced by selected isolates; this result closely agrees with the findings of [78] that ammonia production is commonly demonstrated by isolates from different genera of rhizobacteria as *Bacillus*. It has also been documented that ammonia production has an indirect influence on the

growth of plants. *Bacillus* and *Pseudomonas* were effective producers of ammonia and increased to a significant level the biomass of medicinal and aromatic plant [80]. On the other hand *Azotobacter* isolates have been seen to be less frequent in producing ammonia. Ammonia production has been identified in 95% of the isolates from the rhizosphere of rice; mangrove and soil contaminated by effluent, and influences the promotion of plant growth [78,79].

Different mechanisms of the effective isolates have been observed for their positive performance, such as siderophore, HCN, ammonia, and indole acetic acid (IAA) production, phosphate solubilisation, nitrate reduction, competition for nutrition and space, and hydrolytic enzymes for the inactivation of the pathogen enzymes and degradation of the cell wall. These metabolites were related to suppressing the mycelial growth and spores germination of the pathogen and promoting and stimulating the plant growth [81,82]. It has been reported that bacterial antagonistic and growth promotional activities in the environment is enabled by the production of bacterial allelochemicals, which includes lytic enzymes, iron-chelating siderophores and antibiotics, and plant growth promotion compounds [83,84], thus contributing to sustainable agriculture.

4. Conclusion

Hence, the present study concluded that the isolates of effective bacteria from the different genera, *Alcaligenes*, *Bacillus*, isolated from the rhizosphere of the host plant have the potential to inhibit *F. oxysporum f. sp. melonis*, which causes Fusarium wilt in rock melon. Since they expressed different mechanisms and multifunctional properties against pathogens during their antagonistic activity; by producing antimicrobial compounds, hydrolytic enzymes that attack the cell components of the pathogens and inactivation of pathogen's enzymes as they are the producer of siderophore and HCN, it demonstrates their rhizospheric competitiveness. Moreover, their contribution to crop productivity and the plant growth promotion directly by nitrogen fixation, solubilisation of nutrients and production of phytohormones, or, indirectly, by induced resistance can be proposed to be used as effective biocontrol agents. The productive efficiency of a specific effective microbes may be further enhanced with the optimization and acclimatization according to the prevailing soil conditions. Currently, awareness on application of beneficial microbes as biocontrol agents and biofertilizers is increasing and they are expected to replace the chemical fertilizers, pesticides and artificial growth regulators which have numerous side effects to sustainable agriculture. Further research and understanding of mechanisms of effective microbes mediated-phyto-stimulation would open the way to find out more competent strains which may acts under diverse agro-ecological systems, to maintain soil health and improve crop qualitatively and quantitatively

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Conflict of Interest

The authors have declared that no conflict of interest exists

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