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Biocontrol of *Botrytis cinerea* on Tomato Leaves and Growth Promotion Potential of Soil Isolate HJ505 (*Burkholderia ambifaria*)

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ABSTRACT

Background and Objective: Due to the warm and humid weather in glasshouse or open field in West-Africa, tomato culture is exposed to phytopathogens like *Botrytis cinerea* causing tomato seedlings damping-off, leaves lesions and fruits rot. In this study, Japan soil bacteria HJ505 was evaluated for *Botrytis cinerea* biocontrol and tomato growth promotion. **Materials and Methods:** Dual culture technic was used to evaluate HJ505 inhibitory effect on various pathogens, and microscopy counting used for *Botrytis cinerea* conidia inhibition. Spray technic was used for home Momotaro tomato leaves inoculation, 16S rDNA analyzed for HJ505 identification, disc diffusion used for protease and siderophores production test. HJ505 cells were observed on electron microscopy and volatile compounds production evaluated on divided sealed plates. IAA production by HJ505 was tested using colorimetric method, and broth poisoning technic was used for salinity and acidity tolerance test. Statistical analysis was performed using Student's t-test (two groups), one-way ANOVA followed by Tukey's HSD test (multiple groups), with data presented as Mean±SD and significance considered at $p<0.05$ **Results:** Isolate HJ505 PC1 broth strongly inhibited on plates *Botrytis cinerea* and 5 others tomato fungal pathogens. HJ505 LB broth and filtrate inhibitory effect on pathogen conidial germination were also confirmed. On tomato plants, HJ505 LB broth and filtrate significantly reduced pathogen *Botrytis cinerea* necrotic lesions on leaves. HJ505, identified as *Burkholderia ambifaria*, shared the rod shape of Burkholderia species cells, produced protease and volatile compounds. HJ505 promoted tomato seedling growth, produced siderophores, IAA and was tolerant to pH and salinity variation. **Conclusion:** HJ505 can be considered as a good candidate for *Botrytis cinerea* biocontrol and tomato growth promotion.

KEYWORDS

Botrytis cinerea, *Burkholderia ambifaria*, tomato, soil microbes, biocontrol, growth promotion

INTRODUCTION

Tomato is a vegetable fruit well known in the world and was reported in 2023 as world most harvested vegetable fruit with a production up to 192 millions of tones[1]. Tomato is present in many diets around the world especially in Western Africa countries. In a country like Nigeria, tomato is presented as an important diet heritage and its consumption among a household of seven is estimated at 187 kg per year[2,3]. The

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organisation of local tomato production to meet consumers' high demand has ranked Nigeria as 11th in the world and 2nd tomato largest producer in Africa[3]. However, the threat posed by climate change to the traditional open-field cultivation of tomato in terms of productivity decrease, and susceptibility to pests and diseases, has led to the recommendation of tomato cultivation in controlled conditions such as greenhouse in Nigeria[4]. Another similar suggestion has also been made in Ghana Republic, a neighboring country, confirming the increase of greenhouse tomato production in a near future in West-Africas[5]. Tomato cultivation in general is exposed to many phytopathogens and under greenhouse tomato gray mold caused by *Botrytis cinerea* is well known as big threat to tomato plants but also to the fruits after being harvested[6]. In Burkina-Faso, another West-African tomato-producing country, *Botrytis cinerea* was reported among many others tomato fungal pathogens associated to tomato diseases[7] confirming the presence and the threat of the pathogen in West-Africa. *Botrytis cinerea* is a necrotrophic fungus mainly characterized by its genetic variability and its ability to easily adapt to the environment[8,9]. It was reported infecting various fruits and vegetables like tomato, apple and strawberry[10-12]. To control *Botrytis cinerea*, the use of bacterial biocontrol agents is a sustainable alternative to reduce reliance on chemical pesticides, especially in West Africa, where many phytosanitary chemicals used in agriculture are reported to be harmful to humans and the natural ecosystem 13. *Bulkhoderia* species are gram-negative[13,14] and some are plant pathogens such as *Bulkhoderia plantarii* and *Bulkhoderia glumae*[15]. Some are able to infect animals and humans like *Bulkhoderia mallei and Bulkhoderia. pseudomallei*[16]. *Bulkhoderia ambifaria* used in this study as biocontrol agent, has been reported to inhibit plant pathogens through enzymes and secondary metabolites[17,18]. However inhibitory effect of *Bulkhoderia ambifaria* on tomato leaves infected by *Botrytis cinerea* has not yet been demonstrated. Among Bulkhoderia species, *Burkholderia gladioli* strain KRS027 has been reported inhibiting *Botrytis cinerea* gray mold on tobacco leaves, but not yet on tomato leaves[19]. In this study, Shimane prefecture soil isolated microbe HJ505 will be evaluated against pathogen *Botrytis cinerea* on home Momotaro tomato cultivar (cv.) and also for its growth promotion.

MATERIALS AND METHODS

Study area and duration: The study was conducted from January 2022 to March 2023 in the Plant Pathology Laboratory (*in vitro* and in planta experiment) and Honjo experimental farm (soil microbes isolation) of Shimane University. Both area are located in Matsue city of Shimane prefecture, Southwest Japan.

Plant and pathogens: Home Momotaro tomato cultivar (cv.) seedlings were selected for this experiment and were grown as described by Adam Dade *et al.*[20]. At 21 days old, seedlings were used in the same pot for plant experiments in a growth chamber under a temperature of 26±2°C and 70 % of relative humidity.

Pathogen *Botrytis cinerea* strain MAFF243092 was obtained from the Japan NARO Genebank and was preserved on potato sucrose agar (PSA; 200 g/L potatoes, 2.0% w/v sucrose, 2.0% w/v agar) slants. A diameter of 6 mm mycelial disc was placed on the center of potato dextrose agar (PDA; 200 g/L potatoes, 2.0% w/v dextrose, 2.0% w/v agar) plates at 20±2°C in the glass incubator without light for 3 days and then placed for 7 days under near-ultraviolet radiation provided by fluorescent lamps (FL20S/BLB; Panasonic, Osaka, Japan) at 26±2°C for sporulation. Asexual spores or conidia were collected by pouring 5 mL of sterile distilled water (SDW) onto sporulated PDA culture and gently scratching the conidia into a 10 mL glass beaker with a sterilized spoon spatula. Mycelial solution is then filtered using a 90 mm filter paper (Advantech), and the concentration was fixed to approximately 3×10^6 conidia/mL with a hemocytometer for use. To produce mycelia without sporulation, PDA plates with mycelial disc placed at the center, are kept in the incubation room at 26±2°C in the dark for 14 days.

HJ505 isolation and culture: In this study, four soil samples (HJ1, HJ2, HJ3 and HJ4) were randomly collected from a field on Honjo experimental farm previously used to grow strawberry. From the four soil samples, 100 microorganisms were isolated using a modified method of Lemtukei *et al.*[21]. A little amount

of collected soil samples was placed in a micro tube with 1 mL of distilled water (DW). From each sample 20 µL was spread on humid vitamin acid agar (HVA; 0.1% w/v humic acid, 0.05% w/v Na_2HPO_4, 0.17% KCl, 0.001% w/v $MgSO_4$ $7H_2O$, 0.001% w/v FeSO $7H_2O$, 0.002% w/v CaCO, 1.8% w/v agar and 0.1% v/v of vitamin solution after autoclave). The same amount was also spread on tryptic soy agar (TSA; 1.5% w/v casein pancreatic digest, 0.5% w/v soybean peptic digest, 0.5% w/v NaCl, 1.5% w/v agar). After autoclave, 1% w/v of each cycloheximide and nalidixic acid was added to both medium. All petri plates were incubated for 4 days at 26±2°C. A single colony of HJ505 obtained from both inoculated HVA and TSA media was picked and reinoculated on nutrient broth glucose agar (NGA; 2% w/v nutrient broth, 1% w/v glucose and 1% w/v agar) for 4 days at 26±2°C. Distinct colonies on NGA medium were inoculated into test tubes containing 3 mL of PC1 broth (1 % w/v of each starch, polypeptone, molasses, beef extract at a pH of 7.2). Tubes were then incubated at 180 round per minute (rpm) on a rotary shaker for 7 days at 26±2°C. Isolated microorganisms were suspended in 15-20% glycerol solution and stored at -80°C until being used for *Botrytis cinerea* mycelial growth inhibition screening. For its use, isolate HJ505 glycerol was grown on lysogeny broth agar (LBA; 0.5% w/v yeast extract, 1% w/v bactotryptone, 1% w/v NaCl, 2% w/v agar) and incubated for 3 days at 28±2°C. Colonies were inoculated into culture tubes containing either 5 mL of PC1 or 5 mL of lysogeny broth (LB; 0.5% w/v yeast extract, 1% w/v bactotryptone, 1% w/v NaCl). The culture tubes were incubated at 26±2°C for 3 days at 180 rpm and ready for use. For all experiments, HJ505 PC1 and LB broth with an optical density (OD_{600nm})>2, were used. To obtain the isolate broth filtrate, HJ505 LB broh was centrifuged at 5500 rpm for 25 minutes (min), then filtered through a 0.22-µm pores and used in the experiment.

HJ505 inhibitory test by dual culture: After conducting pathogen inhibition screening using all 100 isolates, among 14 isolates that inhibited *Botrytis cinerea* (data not shown), HJ505 was selected to be used against pathogen in the present study due to its strong inhibitory. The HJ505 was reevaluated for pathogen *Botrytis cinerea* mycelia inhibition using dual culture. Experiment was repeated three times, with five petri plates per treatment. The dual culture experiment was conducted as described by Adam Dade *et al.*[20] Isolate HJ505 inhibitory was evaluated as well against five others tomato fungal pathogens, in dual culture as described by Adam Dade *et al.*[20] The pathogens are *Alternaria alternata*, *Athelia rolfsii*, *Stemphylium lycopersici*, *Fusarium oxysporum* f. sp. *lycopersici*, and *Sclerotinia sclerotiorum*. The area of mycelial inhibition was determined 2 weeks after, using LIA 32 software[20], and the experiment was repeated two times, with five petri plates per treatment.

Inhibition of pathogen germination by HJ505: *Botrytis cinerea* conidia (3×10^6 conidia/mL) suspended in isolate HJ505 LB broth or broth filtrate, was dropped on glass slides and kept in a moist box at 26±2°C. After 24 hrs incubation, the percentage of conidial germination was determined by assessing 50 conidia per drop using light microscopy, counting and following the formula below. The LB broth was used as a control. The experiment was repeated two times, with 6 drops observed per treatment[22].

$$\text{Conidial germination inhibition (\%)} = \frac{\text{Number of germinated conidia}}{\text{Number of assesed conidia per drop}} \times 100$$

Pathogen infection reduction on tomato plant by HJ505: In this experiment, 21 days-old seedlings were used. With a sterilized scissor, five leaves were randomly selected per plant and lightly wounded to facilitate pathogen infection after inoculation. A volume of 6 mL mixed inoculum (1:2) containing 2 mL pathogen *Botrytis cinerea* (3×10^6 conidia/ml) and 4 mL of either HJ505 LB broth or HJ505 LB broth filtrate was sprayed on the leaves of each plant. For the control, 4 mL of LB broth or LB broth filtrate was mixed with 2 mL of pathogen *Botrytis cinerea* (3×10^6 conidia/mL) and sprayed. To obtain LB broth filtrate, LB broth was filtered through a 0.22-µm pores. Five plants were used per treatment and the experiment was

repeated 3 times. After inoculation, the plants were kept in a growth chamber for 3 weeks, then the percentage of infected leaves and infected leaflets, leaflets disease severity index (DSI) and leaflets disease control effect (DCE) were evaluated. To determine the DSI a score previously reported was modified and used[23]. An estimation of the leaflet's infection area was used instead of measuring the lesion diameter. The score was divided from 1 to 5, where 0 means no infection, 1 means 1 to 20% of leaflet surface with necrotic lesions, 2 means 21 to 40%, 3 means 41 to 60%, 4 means 61 to 80% and 5 means 81 to 100%. The formula are described as followed[24,25]:

$$\text{Infected leaves (\%)} = \frac{\text{Infected leaves number}}{\text{Total leaves number}} \times 100$$

$$\text{Infected leaflet (\%)} = \frac{\text{Infected leaflets number}}{\text{Total leaflets number}} \times 100$$

$$\text{DSI (\%)} = \frac{\sum (a \times b)}{N \times Y} \times 100$$

a = Infected leaflets number per plant
b = Infection scale of leaflet
N= Total leaflets number per plant
Y= Highest value on infection scale

$$\text{DCE (\%)} = \frac{\text{Control DSI - Treatement DSI}}{\text{Control DSI}} \times 100$$

HJ505 molecular identification: Isolate HJ505 DNA was extracted, amplified and sequenced following Adam Dade *et al.*[20] using the primers in Table S1. Sequence homology search was conducted with NCBI nucleotide BLAST tool[20]. The function "build" of ETE3 3.1.3[26] was used for alignment and phylogenetic reconstructions as applied on the GenomeNet. Phylogenetic tree was constructed using FastTree v2.1.8 with default parameters[27]. *Escherichia coli*, NBRC102203T was used as outgroup.

Scanning electron microscopy of HJ505: The sample of HJ505 was prepared and scanned under electron microscopy as described by Adam Dade *et al.*[20].

Proteolytic enzyme and volatile compounds production by HJ505: Protease enzyme production was evaluated on skim milk agar (SMA; 0.5% w/v skim milk powder and 1.5% w/v agar). An 8-mm-diameter paper disc (Advantec, Tokyo, Japan) placed in the center of the plate was inoculated either with 50 µL of HJ505 LB broth for the treatment, either with 50 µL of LB broth for the control. The clear area (mm^2) on the SMA plates were measured after 7 days using LIA 32 software[20]. Treatment and control plates are replicated 5 times and the experiment was repeated 3 times. To evaluate the volatile compounds production, a two weeks old mycelium plug was placed on one side of a half divided plate containing PDA medium. For the treatment plate, an amount of 50 µL of isolate HJ505 LB broth was spread on the other side of the half divided plate and for the control plate, 50 µL of LB broth was spread on the other half. Treatment and control plates were replicated 4 times and the experiment was repeated 2 times. The plates were hermetically sealed with Parafilm™ to avoid volatile compounds leaking and were incubated at 26±2°C. The pathogen growth area was measured after 10 days using LIA 32 software[20].

HJ505 growth promotion on seedlings and seeds: Tomato seedlings growth promotion experiment was conducted using a modified method[20]. An amount of 5 mL of HJ505 LB broth was dropped around the base of 21 days-old tomato seedlings on the soil. For the control, 5 mL of LB broth was used. Five plants were used per treatment, and the experiment was done three times. After 3 weeks, the dried weight of the shoots and roots of each 6 weeks-old plant was assessed. For the tomato seeds germination promotion experiment, 10 seeds were kept in culture tube containing HJ505 LB broth for 24 hrs at 140 rpm for treatment use and for the control, 10 seeds were kept in LB broth tube. Among the 10 seeds soaked, one was randomly selected and put 2 mm deeper in SDW-agar glass tube (1% w/v agar) for germination. Treatment and control plates were replicated 5 times and the experiment repeated 2 times. After 1 week, the length of each 7-day-old germinated seed was measured, and the seeds were oven-dried at 70°C until a constant mass to determine the dry weight.

Indole acetic acid (IAA) and siderophores production by HJ505: The production of IAA by HJ505 NB broth and filtrate was evaluated using a modified method[20,28]. Isolate HJ505 LBA colonies were separately inoculated and grown in nutrient broth (NB; 0.5 % w/v peptone; 0.15% w/v yeast extract; 0.15% w/v beef extract; and 0.5% w/v NaCl) without and with 0.1% L-tryptophan (w/v). The cultures tubes were incubated for 4 days at 26±2°C, in a rotary shaker at 180 rpm and later centrifuged at 5500 rpm for 25 min to obtain the supernatant. For HJ505 NB broth, an amount of 1 mL supernatant was collected and mixed with 2 mL of Salkowski's reagent and incubated for 30 min at 23±4°C. For the control, 1 mL of NB broth (with and without L-tryptophan) was mixed with the Salkowski's reagent. For HJ505 NB broth filtrate, the supernatant was filtered through a 0.22-µm pores and 1 mL was mixed with 2 mL of Salkowski's reagent and for the broth filtrate control, 1 mL of NB broth filtered through a 0.22-µm pores was mixed with 2 mL of Salkowski's reagent (with and without L-tryptophan). A spectrometer (UV mini-1240: photometric mode, Shimandzu Co., Kyoto, Japan) was used for absorption measurement at 530 nm. The absorption data of pure 3-Indoleacetic acid standards (Wako Chemical Pure Industries Ltd, Osaka, Japan) set at 5, 10, 20, 50, and 100 µg/mL served as base to assess IAA production using a regression line[29]. Treatments and control were repeated in 5 culture tubes and the experiment was repeated twice. To evaluate HJ505 siderophores production, modified chrome azurol S (CAS) based on a competition for Fe^{3+} between the siderophores and the ferric complex of the Dye CAS was used[30]. For the Blue Dye preparation, fours solutions named 1, 2, 3 and 4 were made. For solution 1, CAS Mordant Blue 29 (0.0605 g) was dissolved in 50 mL of distilled water (DW). For the solution 2, $FeCl_3$-6H2O (0.027 g) and 6N HCl (167 µl) were dissolved in 100 mL of DW. For the solution 3, hexadecyl trimethyl ammonium bromide: HDTMA (0.0729 g) was dissolved in 40 mL of DW. And finally for the solution 4, PIPES-Na (3 g), glucose (2 g) and agar (3 g) were dissolved in 90 mL of N11 medium. To prepare N11 medium, $NaNo_3$ (0.27 g), K_2HPO_4 (0.01 g), $MgSO_4$ (0.045 g), KCl (0.045 g) and $FeSO_4$ (0.0009 g) were dissolved in 90 mL of DW. A respective volume of 5, 1 and 4 mL of solution 1, 2 and 3 were mixed and autoclaved separately with solution 4. After autoclave, the two sterilized solutions were mixed and poured in 7 cm diameter petri plates. HJ505 LB broth drops of 10 µL were placed at four random positions on the CAS medium plates. For the control four drops of 10 µL of LB broth were applied. Treatment and control plates were repeated 3 times and incubated at 26±2°C. After 4 days the orange-yellow coloration area around each spots was measured using LIA 32 software[20]. The experiment was repeated twice.

HJ505 time-lapse sensitivity to salinity and pH variation: For the salinity test, 3 days old HJ505 on LBA was inoculated to LB broth tubes with NaCl concentration adjusted to 0.15 M, 0.3 M, 0.5 M, 1 M, 2 M and 0 M for the control. For the pH test, HJ505 on LBA was inoculated to LB broth tubes with a pH adjusted to 3, 4.2, 5.2, 7.95, 8.95 and 6.82 used as control. Treatments and controls were repeated in 5 tubes and incubated at 26±2°C, in a rotary shaker at 140 rpm. The $OD600_{mn}$ of each tube was recorded at 6, 12, 18, 24 hrs and the experiment was repeated twice.

Statistical analysis: Student's t-test was used to evaluate significance difference in the means values between two groups' data. For multiple groups' analysis, Tukey' Honest Significant Difference (HSD) test run after a one-way ANOVA. SPSS Statistics ver. 30.0 for Windows (IBM, Armonk, NY, USA) was used as statistical software. Data were presented as Mean±Standard Deviation (SD), and differences were considered significant at $p<0.05$.

RESULTS

HJ505 inhibitory test by dual culture: The inhibition area measured with HJ505 treatment was significantly different compared to the control. The HJ505 induced an inhibition area of 2295.33±718.47 mm^2 (Mean±SD) compared to the control which induced 0 mm^2 (Fig. 1).

Inhibition of pathogen conidial germination by HJ505: Pathogen conidia germination evaluated in percentage was strongly and significantly inhibited by HJ505 LB broth and filtrate compared to the control (only LB broth). HJ505 LB broth induced only 3.33±6.95% (Mean±SD) germinated conidia compared to the control which induced 175.17±45.7% (Fig. 2a). The HJ505 LB broth filtrate in the other hand induced 9.67±9.57% compared to the control which induced 237.33±43.6% (Fig. 2b).

Pathogen infection reduction on tomato plant by HJ505: The HJ505 LB broth on home Momotaro cv. tomato leaves significantly reduced infection on leaves and leaflets. The percentages of infected leaves and leaflets induced by HJ505 LB broth were respectively 25.46±22.21 % (Mean±SD) and 12.49±10.48% compared to the control which induced respectively 91.67±14.43% and 60.65±16.81%. The disease severity index (DSI) and disease control effect (DCE) induced by HJ505 LB broth on the leaflets were respectively 7.03±6.49% and 86.13±15.82% compared to the control which induced respectively 55.54±18.61 and 0%. (Fig. 3). Using HJ505 broth filtrate, the percentages of infected leaves and leaflets induced were, respectively 40.11±29.57% and 15.97±13.02% compared to the control which induced respectively 91.22±13.34% and 62.62±19%. The DSI and DCE induced by HJ505 LB broth filtrate on the leaflets were respectively, 10.06±7.55 and 74.86±19.32% compared to the control which induced 50.56±19.89 and 0%. (Fig. 4).

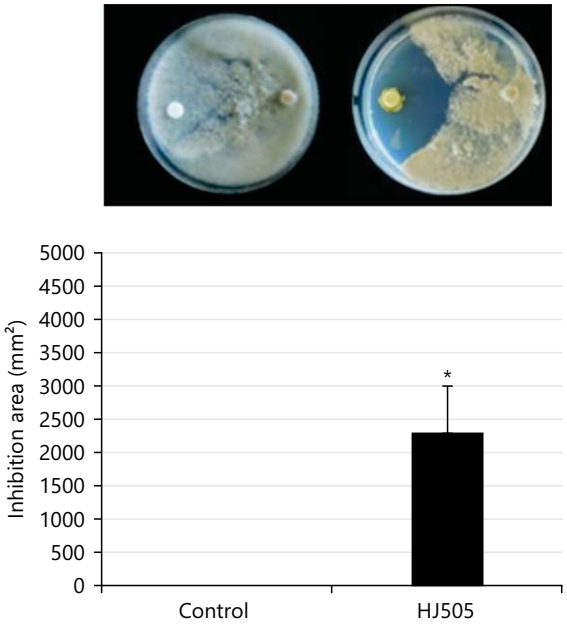

Fig. 1: Inhibition of pathogen mycelial growth by HJ505 PC1 broth using dual culture technic on PDA. Values are means (±SD) of mycelia area

Bars at the top are the standard errors and asterisk indicates a significant difference compared to the control (Student's t-test, $p<0.05$)

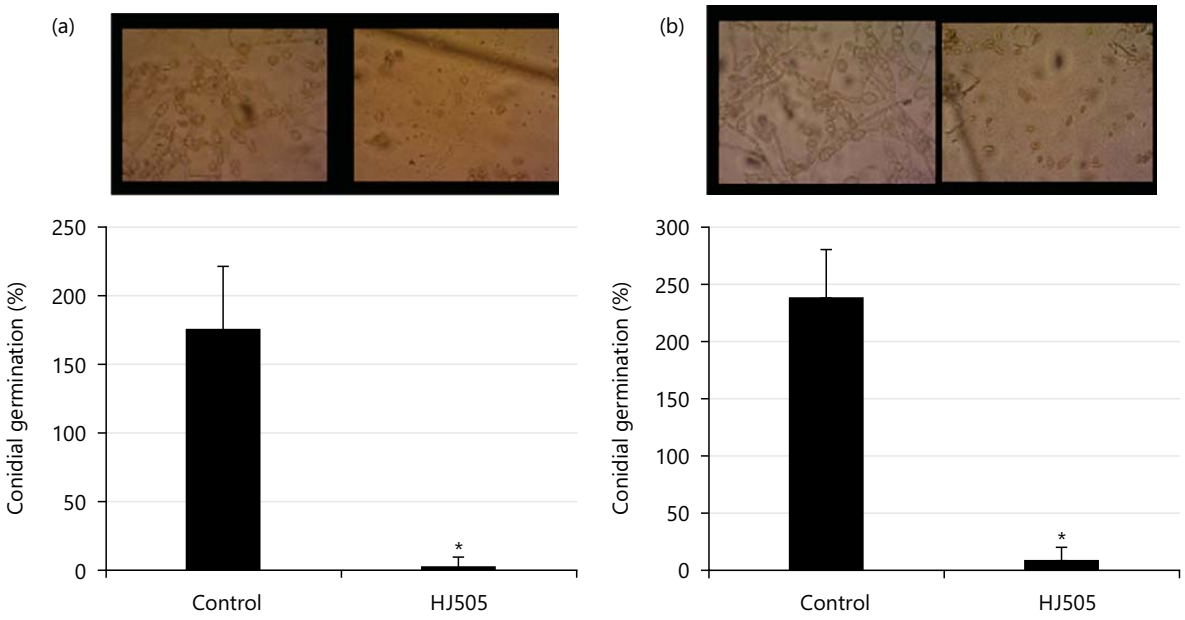

Fig. 2(a-b): (a) Inhibition of conidial germination by HJ505 LB broth and (b) LB broth filtrate. Values are means (±SD) of conidial germination percentage

Bars at the top are the standard errors and asterisk indicates a significant difference compared to the control (Student's t-test, $p<0.05$)

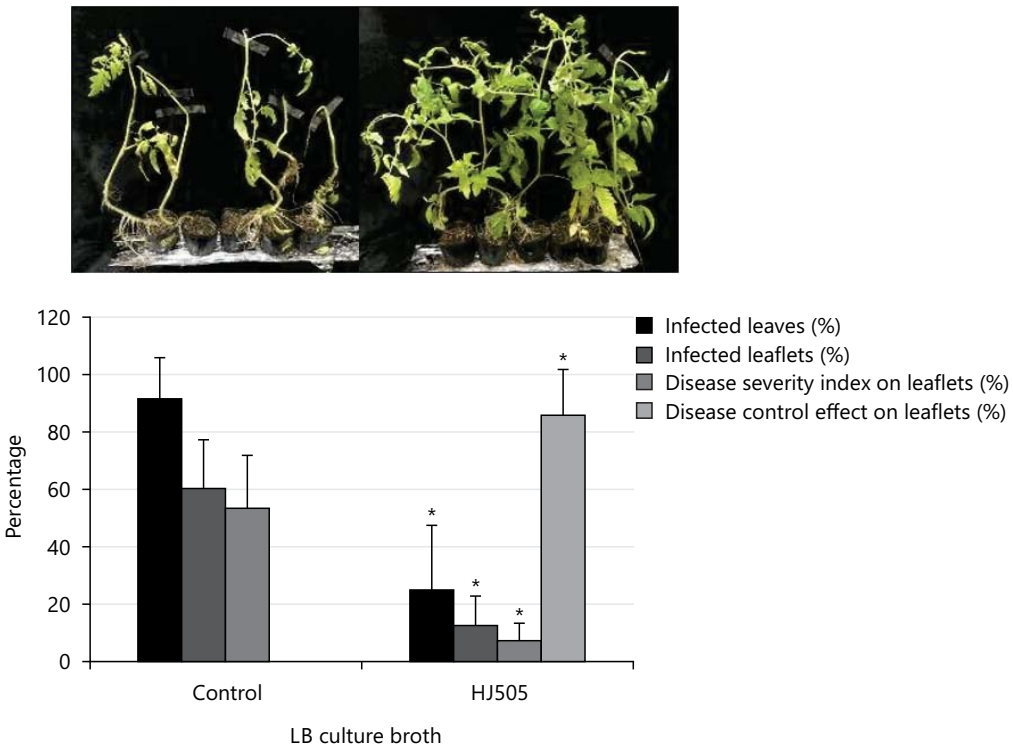

Fig. 3: Inhibition of pathogen infection on home Momotaro cv. tomato leaves by isolate HJ505 LB broth. Values are means (±SD) of leaves and leaflets infection percentage and severity index percentage

Bars at the top are the standard errors and asterisk indicates a significant difference compared to the control (Student's t-test, $p<0.05$)

HJ505 molecular identification: The HJ505 16S rDNA sequence analysis shared 99 % of similarity with *Burkholderia ambifaria* and the phylogenetic tree was constructed (Fig. 5). The 16S rDNA sequence has been deposited in the DDBJ/EMBL/GenBank database under accession number LC909675, with a full length of 1455 bp.

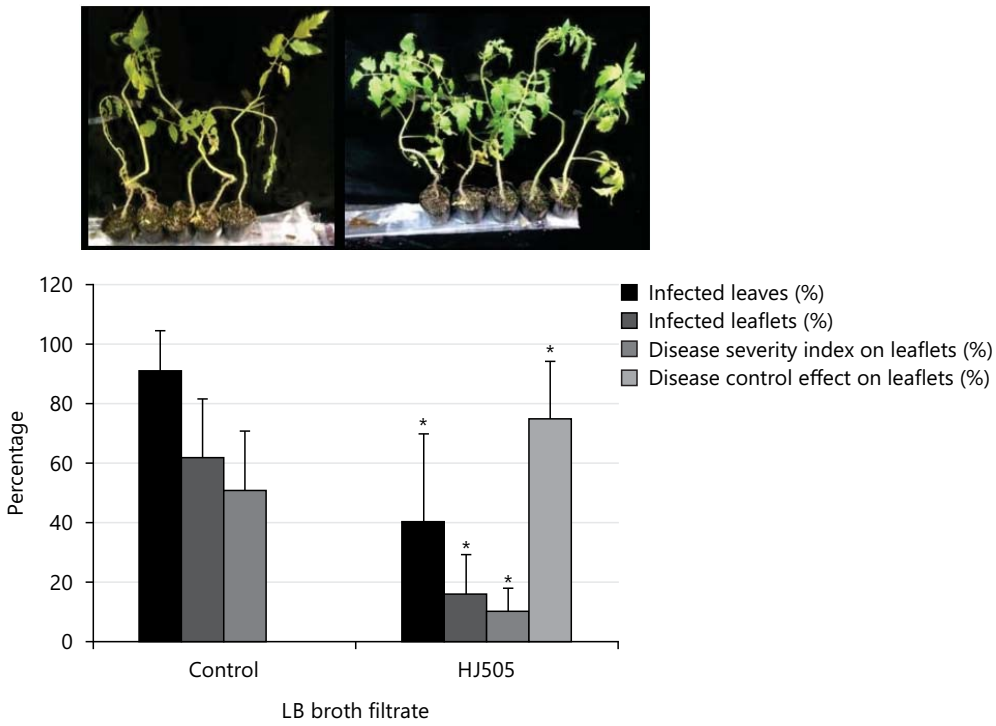

Fig. 4: Inhibition of pathogen infection on tomato home Momotaro cv. leaves by isolate HJ505 LB broth filtrate. Values are means (±SD) of leaves and leaflets infection percentage and severity index percentage

Bars at the top are the standard errors and asterisk indicates a significant difference compared to the control (Student's t-test, $p<0.05$)

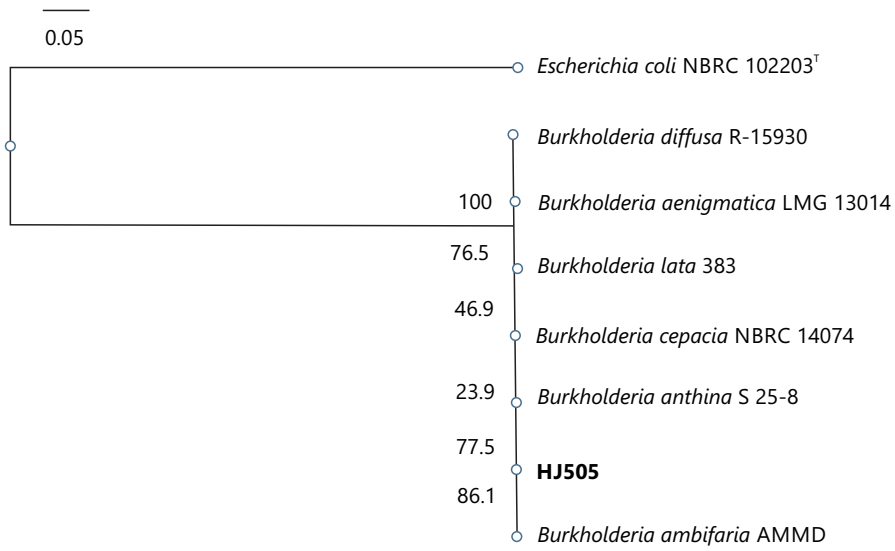

Fig. 5: Phylogenetic tree based on 16S rDNA sequences from *Burkholderia* species and isolate HJ505. *Escherichia coli* was used as outgroup and scale bar represent 5% sequence dissimilarity

Scanning electron microscopy of HJ505: *Burkholderia* species, straight rod shaped, was confirmed after scanning HJ505 cells and the dividing process was observed. The size of the cell was approximately 0.3-0.5×0.6-1 μm (Fig. 6).

Proteolytic enzyme and volatile compounds production by HJ505: The HJ505 protease production was evaluated through the clear area around the disc paper inoculated with HJ505 LB broth. HJ505 induced a clear area of 1726.12±293.0 mm^2 (Mean±SD) significantly different to the control which induced

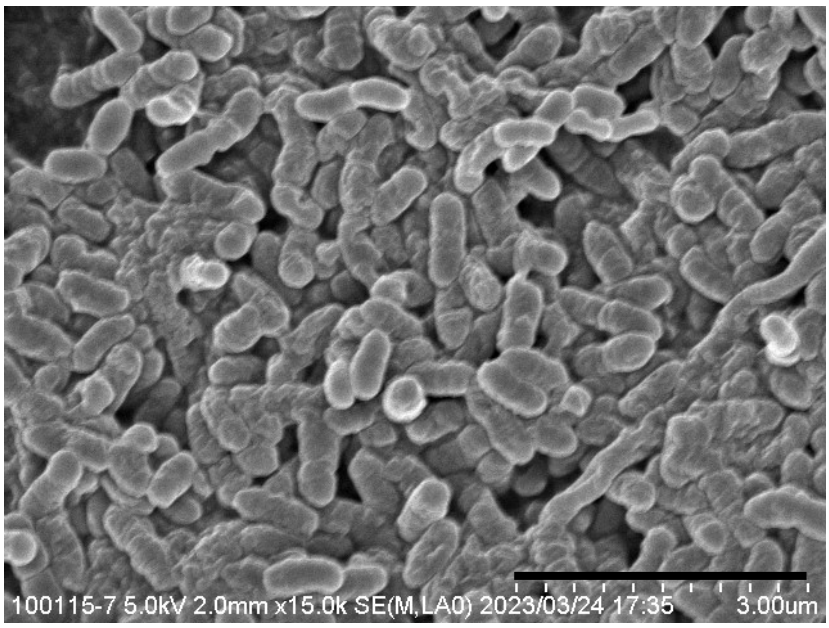

Fig. 6: Isolate HJ505 cells scanning electron micrograph on LBA medium
Scale bar = 3 µm

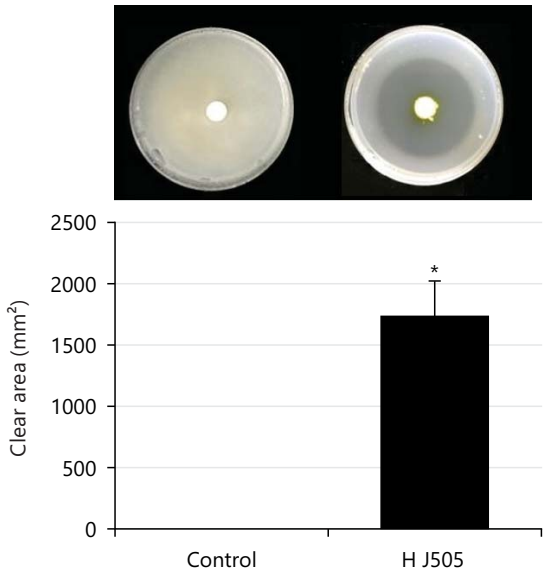

Fig. 7: Protease production by HJ505 LB broth on skim milk agar. Values are the mean (±SD) of clear area Bars at the top are the standard errors and asterisk indicates a significant difference compared to the control (Student's t-test, $p<0.05$)

0 mm^2 (Fig. 7). The production of volatile compounds by HJ505 LB broth was also confirmed by observing pathogen mycelial growth inhibition. Mycelia area of pathogen after HJ505 inoculation was 125.04±43.26 mm^2 compared to the control which induced 2147.29±321.46 mm^2 (Fig. 8).

Inhibition of 5 others tomato fungal pathogens by HJ505: Isolate HJ505 PC1 broth significantly reduced 5 others tomato pathogen mycelia growth compared to the control. In the presence of HJ505 PC1 broth, the mycelia growth area of *Fusarium oxysporum* f. sp. *Lycopersici*, was 987.61±249.96 mm^2 (Mean±SD) compared to the control (presence of PC1 broth only) which induced 3092.11±74.14 mm^2. The mycelia growth area of *Athelia rolfsii* was 2767.67±132.01 mm^2 compared to the control which induced 3119.83±10.67 mm^2. The mycelia growth area of *Sclerotinia sclerotiorum* was 1936.14±108.14 mm^2

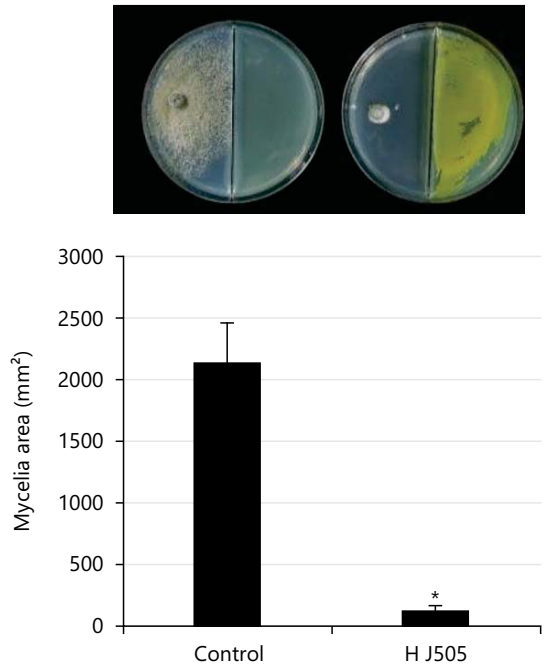

Fig. 8: Volatile compounds production by isolate HJ505 LB broth. Values are means (±SD) of mycelia growth area

Bars at the top are the standard errors and asterisk indicates a significant difference compared to the control (Student's t-test, $p<0.05$)

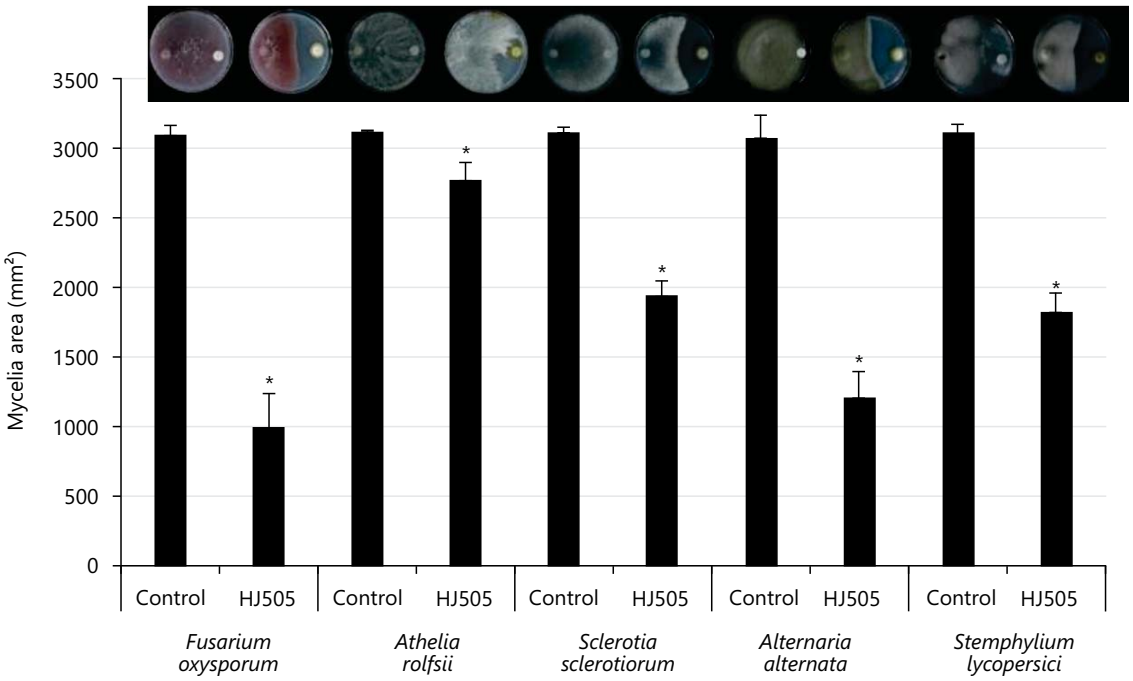

Fig. 9: Inhibition of 5 others tomato fungal pathogens by isolate HJ505 PC1 broth. Values are means (±SD) of mycelia growth area

Bars at the top are the standard errors and asterisk indicates a significant difference compared to the control (Student's t-test, $p<0.05$)

compared to the control which induced 3113.51±35.95 mm^2. The mycelia growth area of *Alternaria alternata* was 1204.59±195.92 mm^2 compared to the control which induced 3071.44±168.10 mm^2 and finally for *Stemphylium lycopersici*, the mycelia growth area was 1821.03±140.83 mm^2 compared to the control which induced 3103.39±67.95 mm^2 (Fig. 9).

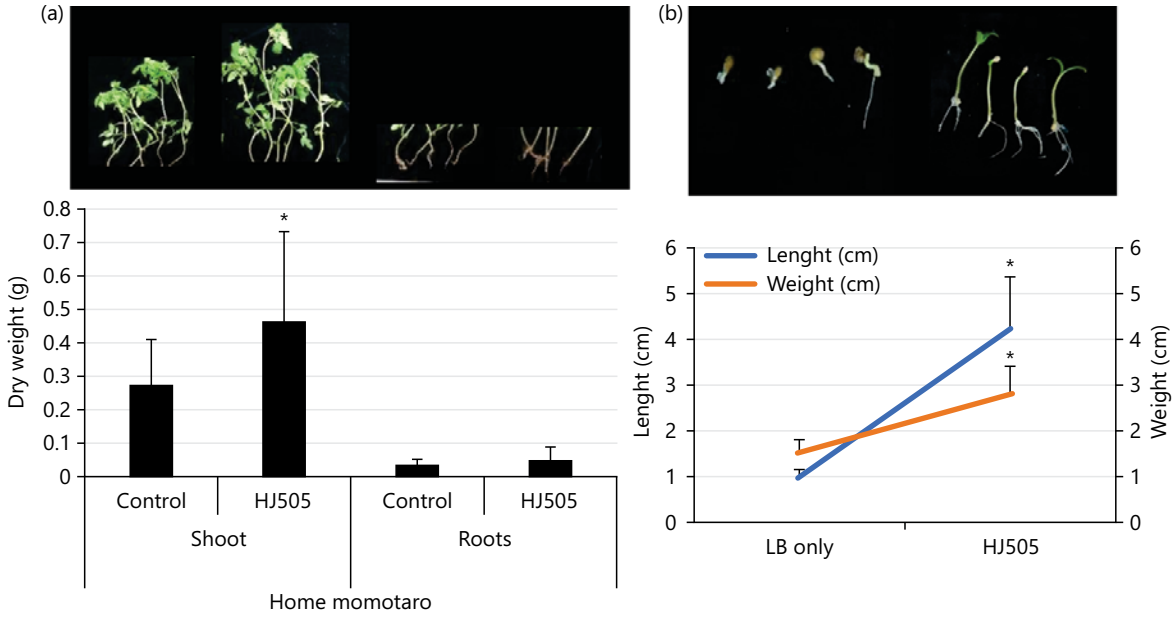

Fig. 10(a-b): (a) Growth promotion on home Momotaro cv. seedlings and (b) Seeds germination (b) Using isolate HJ505 LB broth. (a) Values are means (±SD) of 6 weeks old home Momotaro tomato plants dry weight and (b) One week old seedlings length and weight

Bars at the top are the standard errors and asterisk indicates a significant difference compared to the control (Student's t-test, $p<0.05$)

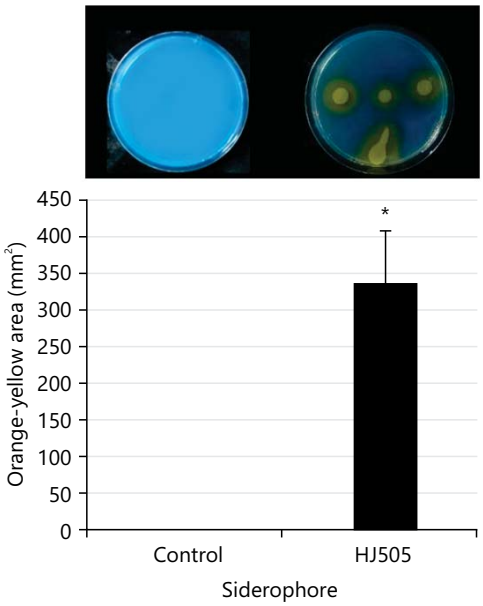

Fig. 11: Siderophores production by isolate HJ505 LB broth. Values are means (±SD) of orange-yellow area

Bars at the top are the standard errors and asterisk indicates a significant difference compared to the control (Student's t-test, $p<0.05$)

HJ505 growth promotion on seedlings and seeds: The growth promotion of HJ505 LB broth on tomato home Momotaro cv. seedlings was more effective on leaves than roots. On the leaves, the dry weight induced was 0.46±0.27 g (Mean±SD) significantly different to the control (only LB broth) which induced 0.27±0.13 g. On the roots, the dried weight induced 0.05±0.04 g and was not significantly different to the control which induced 0.03±0.02 g (Fig. 10a). The seeds germination and growth were also highly promoted by HJ505 LB broth. The length induced are 4.24±1.11 cm significantly different to the control which induced 0.98±0.17 cm and the weight induced are 2.8±0.61 mg significantly different to the control which induced 1.51±0.29 mg (Fig. 10b).

Table 1: IAA production by isolate HJ505 LB broth

Treatments	IAA values ppm
Control	0.00±0.00[a]
HJ505 without tryptophan	33.67±5.20[b]
HJ505 with tryptophan	92.78±2.47[c]

Values represent were Mean±SD and Different letters within a column indicate significant difference (Tukey HSD test, p<0.05)

Table 2: IAA production by isolate HJ505 LB broth filtrate

Treatments	IAA values ppm
Control	0.00±0.00[a]
HJ505 without tryptophan	0.00±0.00[a]
HJ505 with tryptophan	19.91±2.74[b]

Values represent were Mean±SD and Different letters within a column indicate significant difference (Tukey HSD test, p<0.05)

Table 3: Isolate HJ505 sensitivity to salinity timelapse in cell optical density (OD_{600})

	NaCl					
Hours	0 M	0.15 M	0.3 M	0.5 M	1 M	2 M
	HJ505 (OD_{600}), (mean±SD)					
6	0.56±0.08	0.58±0.06	0.47±0.06	0.48±0.07	0.33±0.06	0.03±0.01
12	1.67±0.11	1.79±0.09	1.19±0.26	0.41±0.25	0.17±0.08	0.03±0.01
18	2.09±0.03	2.08±0.04	2.09±0.03	1.75±0.15	0.77±0.1	0.03±0.01
24	2.09±0.03	2.09±0.03	2.09±0.03	1.62±0.13	0.24±0.1	0.03±0.01

Table 4: Isolate HJ505 sensitivity to pH timelapse in cell optical density (OD_{600})

	pH					
Hours	6.82	3	4.20	5.20	7.95	8.95
	HJ505 (OD_{600}), (mean±SD)					
6	0.9±0.19	0.23±0.08	0.54±0.1	0.76±0.07	0.31±0.04	0.57±0.06
12	1.69±0.12	0.33±0.8	0.66±0.1	1.8±0.15	0.67±0.15	0.27±0.25
18	2.07±0.05	0.16±0.05	1.02±0.28	2.09±0.03	1.15±0.51	0.35±0.14
24	2.08±0.04	0.03±0.03	2.09±0.03	2.08±0.04	2.09±0.03	0.02±0.01

Indole acetic acid (IAA) and siderophores production by HJ505: HJ505 NB broth IAA production was doubled with tryptophan. The HJ505 NB broth without tryptophan produced 33.67±5.20 ppm (Mean±SD) significantly different to 0 ppm for the control (only NB broth) and HJ505 NB broth with tryptophan produced 92.78±2.47 ppm significantly different to 0 ppm for the control (only NB broth with tryptophan). The statistical analysis are presented in the Table 1. For HJ505 NB broth filtrate, IAA was low with tryptophan and was not produced without. The HJ505 NB broth filtrate with tryptophan produced 19.91±2.74 ppm significantly different to 0 ppm for the control (only NB broth filtrate with tryptophan) and without tryptophan, HJ505 NB broth filtrate produced 0 ppm compared to 0 ppm for the control (only NB broth filtrate). The statistical results are presented in Table 2. As for siderophores production, isolate HJ505 LB broth induced an orange-yellow coloration area of 336.09±70.85 mm^2 significantly different to 0 mm^2 for the control (Fig. 11).

HJ505 timelapse sensitivity to salinity and pH variation: The sensitivity to salinity and pH variation data of isolate HJ505 in LB broth are presented in Table 3 and Table 4. As for the salinity sensitivity, HJ505 in LB broth at 0 M of NaCl was considered as control. HJ505 in LB broth at 0.15 M, at 0.3 M of NaCl and the control presented a linear growth and reached their maximal growth expressed in cells density ($OD_{600>}2$) at 18 hrs after inoculation (Table 3). At this same duration, the growth of HJ505 in LB broth at 0.5 M of NaCl, was slightly lower compared to the control and significantly different. From 6 to 24 hrs after inoculation, the growth of HJ505 in LB broth at 1 M of NaCl was very low, significantly different to the

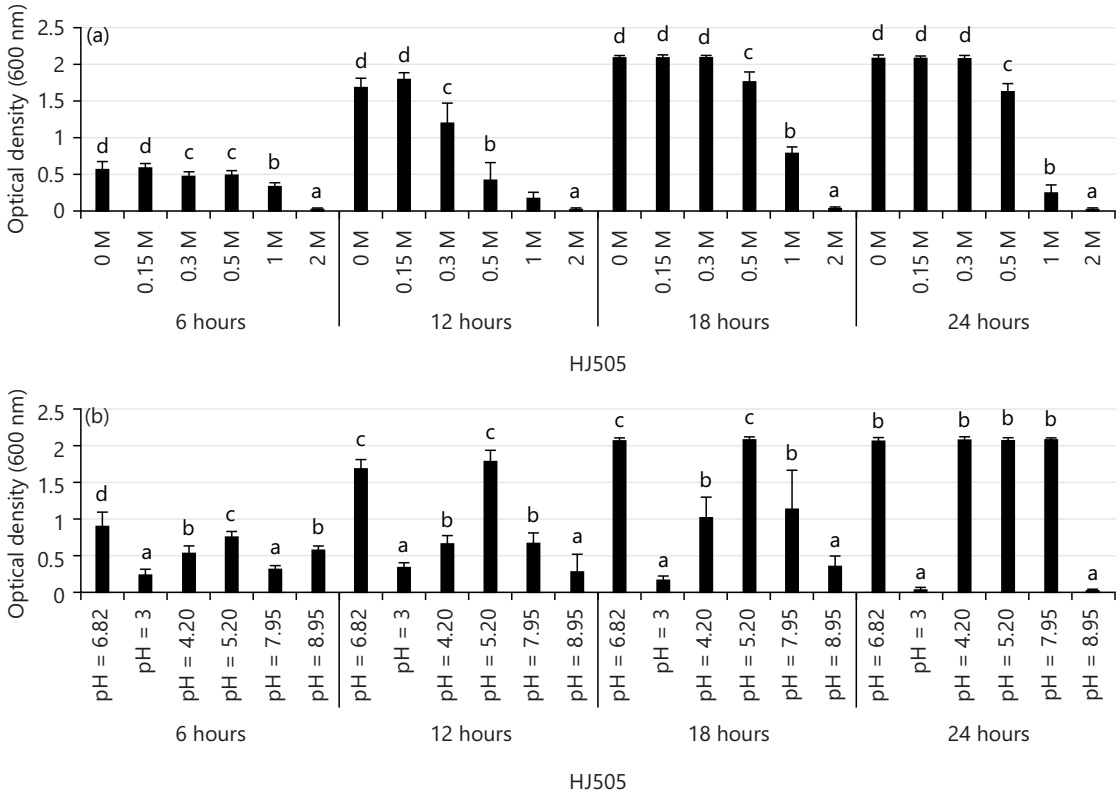

Fig. 12(a-b): (a) Isolate HJ505 sensitivity to salinity and (b) pH variation. Values are means (±SD) of optical density (OD_{600nm})

Bars at the top are the standard errors and letters indicate significant difference (Tukey HSD test, $p<0.05$)

control and no growth was recorded at 2 M (Fig. 12a). As for the pH sensitivity, HJ505 in LB broth with a pH of 6.82 was considered as control and had its maximal growth expressed in cell density ($OD_{600}>2$) at 18 hrs after inoculation (Table 4). Similar to the control, only HJ505 in LB broth with a pH of 5.2 reached its maximal growth at 18 hrs after inoculation ($OD_{600}>2$). HJ505 in LB broth with a pH of 4.2 and 7.95 had a lower growth significantly different to the control and finally reached their maximal growth at 24 hrs after inoculation ($OD_{600}>2$). At a pH of 3 and 8.95, HJ505 growth was constantly low from 6 to 18 hrs after inoculation and no growth was recorded at 24 hrs after inoculation. (Fig. 12b).

DISCUSSION

Control strategies against plant pathogenic fungi mainly involve the use of chemical fungicides which has led to development of resistance to these chemicals[31]. Therefore, it is necessary to develop a sustainable way of disease management like the use of microbial fungicides which are safe and environment friendly[32]. The rod shaped of microbial fungicide *Burkholderia ambifaria* HJ505 scanned in this study was similar to same genus *Burkholderia gladioli* strain KRS027[19]. In this study, *Burkholderia ambifaria* inhibitory effect on pathogen *Botrytis cinerea* mycelia growth is not yet reported. However same genus *Burkholderia gladioli*[19] and *Burkholderia plantarii* BpMS90[33] inhibitory on *Botrytis cinerea* mycelia growth was confirmed. The biocontrol and reduction of *Botrytis cinerea* necrotic lesions on tomato leaves by *Burkholderia ambifaria* has not yet been reported however cepacin metabolite produced by *Burkholderia ambifaria* has been identified to be responsible of *Burkholderia ambifaria* antagonistic potential against pathogens[17]. Same genus, *Burkholderia plantarii* BpMS90 has been reported inhibiting *Botrytis cinerea* symptoms on tomato fruits and 10 others plant pathogens *in vitro*[33] aligning with various fungal pathogen inhibited by HJ505 in this study. About the growth promotion of isolate HJ505 this is the first growth promotion report of *Burkholderia ambifaria* on tomato. However, the growth promotion of barley seedlings[34] and wheat[35] by *Burkholderia ambifaria* T16 was confirmed through various metabolites like IAA, siderophores,

phosphate-solubilization, 1-aminocyclopropane-1-carboxylate. It has been demonstrated that *Burkholderia ambifaria* produced many structures of siderophores such as PCH, ornibactin (with antimicrobial properties), malleobactin, cepaciachelin, azurechelin and cepabactin[14]. These capacity of siderophores production can allow *Burkholderia* sp to survive in iron deficient environment. Another specie, *Burkholderia gladioli* strain KRS027 also produced siderophores, protease and inhibited pathogen *Botrytis cinerea* with its volatile compounds[19]. *Burkholderia ambifaria* strain H8 was also reported to inhibit pathogen *Fusarium graminearum* causing maize stalk through its volatile compounds dimethyl disulfide[36] aligning with HJ505 volatile compounds secretion confirmed in the present study. *Burkholderia ambifaria* strain T16 has been reported degrading fusaric acid mycotoxin produced by *Fusarium* species pathogen causing wilts and root rot diseases on variety of plants and also reducing disease infection on the barley seedlings plant[34]. Furthermore, *Bacillus* spp. and *Pseudomonas* spp., well known biocontrol agents, have failed to degrade fusaric acid mycotoxin confirming the strong antifungal properties of *Burkholderia ambifaria* HJ505 in the reduction of gray mold infection on tomato leaves in this study. The tolerance of *Burkholderia ambifaria* to low pH has also been demonstrated[34,37] and it was suggested that its use as probiotic could contribute to human health management. This study confirmed the possible use of HJ505 in West-African ecosystem characterized by warm and humid climates as well as various acid and saline soils. Furthermore, the stability of isolate HJ505 to temperature, pH and salinity is a significant advantage favoring its validation and commercialization as new biopesticide. In conclusion, *Burkholderia ambifaria* strain HJ505 has the potential to become a good biocontrol agent, and contribute to the development of a new microbial fungicide to prevent tomato gray mold caused by *Botrytis cinerea*. This study aligned with the findings of Adam Dade *et al*.[20] as research conducted in Japan and using soil ecosystem microbes for a sustainable control of diseases seen as a threat in West-African agriculture. It's also the first report on *Burkholderia ambifaria* inhibitory effect on *Botrytis cinerea* gray mold on tomato leaves.

CONCLUSION

This study investigated the biocontrol and growth-promoting potential of soil bacterial isolate HJ505 against tomato gray mold pathogen *Botrytis cinerea* using accessible methods to identify isolate HJ505 from cultivated soil; evaluate its inhibitory activity both *in vitro* and in planta; determine its scientific identity; assess its plant growth–promoting properties; identify primary and secondary metabolites involved in biocontrol and growth promotion and examine isolate HJ505 sensitivity to environmental factors specific for future application from Japan to West-Africa. This study could encourage African researchers, particularly in West Africa, to conduct more identification and characterization studies on local soil microbial isolates with biocontrol and growth-enhancing potential. These efforts will facilitate the selection of the most promising biocontrol agent for developing affordable antagonist-based biopesticides.

SIGNIFICANCE STATEMENT

This study is in phase with the second and third sustainable development goals aiming zero hunger, good health and well-being particularly in underdeveloped countries. A low-cost sustainable management of cultured plant diseases and fertilization using natural bioresources like soil microbes is a key point to reduce in the short term and stop in the long term the use of chemical pesticides and fertilizers. This study results highlighted the inhibitory potential of Japan soil bacteria *Burkholderia ambifaria* HJ505 against pathogen *Botrytis cinerea* seen as threat in West-Africa tomato production. It has also highlighted the significant promotion effect on tomato seed germination and seedling growth. As a previous step to its application in West-Africa, the tolerance of Japan isolate HJ505 to salinity and acidy very common in West-African soils confirmed the possible use in others agroecosystems. This study presented a set of useful methods for beneficial local soil microbes' isolation and evaluation for inhibitory and growth promotion potential.

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SUPPLEMENTARY DATA

Table S1: The sequence of Primers used for the isolate HJ505 molecular identification.

Primers	Sequence (5′ → 3′)	Reference
fD1	AGAGTTTGATCCTGGCTCAG	Huong *et al.*[38]
rP2	ACGGCTACCTTGTTACGACTT	Huong *et al.*[38]
EUB906F	AAACTCAAAGGAATTGRCGG	Matsui *et al.*[39]
EUB532R	CACGGTCGKCGGCGCCATT	Matsui *et al.*[39]
1115R	GTTGCTCGCGTTGGGA	Lanoot *et al.*[40]
802R	CCTAATCTATGGGACCAT	Lanoot *et al.*[40]
926F	AAACTCAAAGGAATTGACGG	Lanoot *et al.*[40]
785F	GGATTAGATACCCTGGTAGTC	Lanoot *et al.*[40]
536R	GTCGTCGGCGCCATTATG	Lanoot *et al.*[40]