Defense Biopriming and Antimicrobial Activity of Endophytic Bacteria and Associated *Bacillus* Species Contribute to Bacterial Crown Rot Tolerance in Papaya

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Running Head: Papaya bacterial crown rot tolerance

Bacterial crown rot (BCR) is a re-emerging disease of papaya (*Carica papaya*) in Southeast Asia caused by a gram-negative, facultative anaerobic bacterium, known as *Erwinia mallotivora*. Ultrastructural observations using SEM revealed colonization of bacterial community common in a BCR-tolerant genotype, before onset of tisue regeneration or 'regrowth'. This observation led to the hypothesis that papaya-associated bacteria might play a role in BCR tolerance. Isolation, characterization and identification using 16S rRNA sequence of bacteria associated with the tolerant 'regrowth' genotype resulted to the discovery of two endophytic bacteria, of genera *Kosakonia* (isolate EBW) and *Sphingomonas* (isolate EBY). Papaya root-associated *Bacillus* isolates were also identified to belong to the *B. amyloliqufaciens* clade (isolate BN, BS) and *B. thuringiensis* clade (isolate BT). Separate inhibition assays indicated that, papaya latex and autoclaved culture metabolites (ACMs) from isolated bacteria promptly (between 18-30h) and efficiently inhibited (60-65%) *E. mallotivora* proliferation *in vitro*. Moreover, when ACMs from BN and EBW were inoculated in surface-sterilized papaya seeds, it variably retarded seed germination (20-60% reduction), depending on plant genotype, but significantly stimulated plant growth and biomass accumulation, at around two-fold increase. Interestingly, ACMs from all isolates, especially EBW, significantly reduced BCR incidence and severity in susceptible genotype at around two-fold. Finally, these results indicated that tolerance of papaya against *E. mallotivora* infection is probably influenced by host innate properties such as tissue regrowth and pathogen inhibition by plant latex, and acquired properties including pathogen antagonism, growth promotion and defense biopriming through metabolites from beneficial endophytic and root-assocaited bacteria.

Keywords: bacterial crown rot, defense biopriming, *Bacillus,* endophytic bacteria

Introduction

Papaya (*Carica papaya*) is an economically important tropical fruit, mainly cultivated for food and cosmetic products. Due to its popularity, production of papaya steadily increased by 4.35% (2002-2010) (Evans and Ballen, 2012). However, the industry still faces yield losses due to diseases, causing problems from plantation, transportation and processing until marketing (Ventura *et al*., 2004). Most importantly, emerging diseases greatly impair papaya production, and even cause up to 100% yield loss in some afflicted areas, especially in Asian tropical countries (Ploetz, 2004). A re-emerging disease the of papaya, named bacterial crown rot (syn. soft rot, canker, decline) caused by a bacterium of genus *Erwinia* was reported in the Southeast Asian region (Maktar *et al.,* 2008). Historically, the disease was reported in Java (von Rant, 1931), and in other regions such as Mariana Islands (Trujillo and Schroth, 1982) and in the US Virgin Islands (Webb, 1985). In Mindoro, Philippines, a similar soft rot disease, which has water-soaked symptoms on leaves, petioles and whorl accompanied by a foul odor was reported. The pathogen was identified under the genus *Erwinia* (Pordesimo and Quimio, 1965).

Re-emergence of *Erwinia* rot in papaya was reported in many regions across the globe in the past decade. In early 2000, the bacterial 'canker' disease of papaya was reported in the Mediterranean, caused by a bacterium belonging to the genus *Erwinia*. The study then proposed a new species named *Erwinia papayae* sp. nov. (Gardan *et al*., 2004). Halfway across the globe, a bacterium identified as *Erwinia papayae* was reported to cause 'papaya dieback' disease in Malaysia (Maktar *et al*., 2008). Interestingly, the 'crown rot' disease of papaya in the Republic of Tonga in Africa was reported to be caused by a related species, *E. mallotivora* (Fullerton *et al*., 2011). In Asia, the crown rot disease that was recently observed in the Philippines was also caused by *E. mallotivora* (dela Cueva *et al*., 2012). *E. mallotivora* can be robustly distinguished from *E. papayae* by physiological tests such as sugar utilization and catalase production or through phylogenetic analysis using 16S rDNA sequence (Amin *et al*., 2011).

Interestingly, in the Philippines, studies were reported regarding the possible resistance or tolerance of papaya against the bacterial crown rot disease. A slow hypersensitive response followed by tissue hardening and lateral stem regrowth at the junction of the diseased and healthy stem was observed in some genotypes. The phenomenon is simply referred to as 'regrowth' and is thought to be the manner by which papaya recovers from the disease, and perhaps a mechanism for resistance or tolerance (Magdalita *et al*., 2016). Breeding for resistance and development of management strategies against the bacterial crown rot disease are already in its early stages in the Philippines. Regrowth hybrid lines with good marketable qualities and with resistance to papaya ringspot disease are currently under preliminary field trials and back-cross F3 progenies are now in the pipeline (Magdalita *et al*., 2015).

However, there are no reports yet regarding the events in pathogenesis up to the recovery of papaya from the crown rot disease. Regrowth tissue examination was done initially in this study to gain insight to probable role of beneficial microbes in disease tolerance. This was followed by antimicrobial assays using papaya latex, culture metabolites from bacterial endophytes and root-associated microbes and priming for disease resistance. Antimicrobial activity and defence biopriming of beneficial microbes were thought to be acquired mechanisms for pathogen defense in plants based on previous studies (El Moussaoui *et al*., 2001; Podolich *et al*., 2015; Liu *et al*., 2017; Song *et al.,* 2017). The use of these augmentative defense strategies in as early as seed or seedling stage would help in developing more resilient genotypes that are otherwise highly susceptible to disease, but with good marketable qualities.

Materials and Methods

Ultrastructural Observation of Papaya Regrowth Tissues

Tissue samples from healthy, infected and regrowth two-month old plants were prepared and examined. The varieties used were genotype 5648 (var. *Cariflora*) for the healthy and regrowth, and genotype 501 (var. *Solo*) for the susceptible. Three plants for each tissue type were randomly sampled in a greenhouse experiment for crown rot resistance screening, wherein three stem portions were obtained for ultrastructural analysis. A 3-mm thick cross section of the stem were made using a #10 sterile surgical blade followed by fixation in 2.5% glutaraldehyde solution in phosphate buffer for 2 h in ice. After fixation, the samples were washed 3-5 times in phosphate buffer solution (pH 7.2). Post-fixation processing was done using 1% osmium tetroxide solution in phosphate buffer for 1-2 h followed by 3-5 times washing in phosphate buffer. The samples were subjected to dehydration series of increasing ethanol concentration (50-70-80-90-95-100%) in ice, and final solution of 100% ethanol at room temperature.

The samples were mounted on sample stubs before proceeding to the next step. The samples were subjected in a two-step substitution with isoamyl acetate at 50% and 100% solution, immediately followed by air drying with hexamethyldisilazane (HMDS). Lastly, ion coating was done using palladium particles to make the samples ready for SEM imaging. Imaging was done using the Phenom XL™ (Phenom-World BV, Eindhoven, The Netherlands), a table top scanning electron microscope. The samples were subjected to either 10 or 15 kilovolts (kV) with magnification ranging from 500X (surface) to 35000X (ultrastructure). Images were captured with the full backscatter detection (BSD) mode. The images captured were saved as TIFF files ready for examination.

Isolation of Endophytic Bacteria and *Bacillus* species in Regrowth Tissues

Stem portions from the regrowth plants were directly obtained from the junction of the regrowth and the healthy stem part. Similarly, portions of the roots of regrowth plants tissues were prepared. Separately, the tissues underwent surface sterilization following the procedure by Coombs and Franco (2003), with some modifications. The procedure is as follows: 95% ethanol for 1 min, 3% sodium hypochlorite solution for 6 min and 95% ethanol for 0.5 min followed by rinsing twice with sterile distilled water for 5 min (Coombs and Franco, 2003). The sterilized tissues underwent a 10 min ooze test in 5 mL of sterile water in a test tube to visibly detect bacterial colonies oozing out to the medium. A loopful of the bacterial ooze suspension was streaked onto peptone sucrose agar (PSA) plates and incubated at room temperature for 24 h. Single colonies of actively growing bacteria were transferred to new PSA plates for subculture and purification. Pure colonies of the bacteria were maintained in PSA culture slants or as glycerol-skim milk stocks and stored at 4ºC for future use.

Cultural Characterization and Pathogenicity Tests of Bacterial Isolates

Five different types of culture media were prepared in triplicates: potato dextrose peptone agar (PDPA), peptone sucrose agar (PSA), King's medium B agar (KMB), yeast extract dextrose-calcium carbonate agar (YDCA) and tetrazolium chloride agar (TZCA). A loopful of a very diluted suspension of the bacterial isolate was streaked multiple times onto the surface of the culture medium to produce single colonies that are very well-separated after about 7 d of incubation at room temperature. The color, diameter of the colony, shape (e.g. shape, surface

and elevation), consistency and pigmentation of the colonies were noted. A single stroke agar colony of the bacterium was also characterized. Test tube slants of PDPA and nutrient agar (NA) were prepared. A single stroke of an inoculating needle dipped in a single colony of the bacterium was made onto the surface of the medium. The colony was described after one week of observation. Colony color, growth, odor, chromogenesis and other characteristics were noted. Additionally, growth in nutrient broth was characterized. A loopful of the bacterium's colony was obtained and suspended in nutrient broth (NB) in a test tube. Observations were done after one and two weeks. Lastly, the morphology of the bacterium was characterized using gram staining methods and the average diameter of the colony was also estimated after one week of incubation.

In order to test for pathogenicity of the bacterial isolates, suspensions of the bacteria grown PSA slants were used in inoculation of 2 month old seedlings at a concentration of 10^8 cfu/mL. BCR-susceptible genotype 336 was used as the test plant. The plants were wounded first at the leaf petiole and leaf midrib using a sterile needle. The inoculum was delivered using a sprayer at approximately 1.0 mL per plant. Observations were done at 7 and 21 d after inoculation. Successful infection is indicated by necrosis and water-soaked lesions and progressive rotting from the point of wounding.

Biochemical Tests, DNA Extraction and Aplification of 16S rDNA

Biochemical tests include assay for catalase, cysteine desulfurase, lipase and gelatinase activities, while physiological test include test for oxygen requirement and utilization of starch and other sugars. All tests conducted in this part of the study were adapted from the procedures in major phytobacteriology books (Goszczynska *et al*., 2000; Jense, 2005; Borkar, 2018), with minor modifications. Initial characterization and identification based on biochemical tests were done using the *Bergey's* Manual of Systematic Bacteriology (Brenner *et al.*, 2009). The tests were done in triplicates and the entire experiment was repeated twice to verify initial results.

DNA extraction was done using a simple DNA extraction protocol as reported by Chen and Kuo in 1993, with some modifications. Single pure colonies were harvested in 72-h cultures in peptone sucrose agar (PSA) slants. A loopful of the bacterial colony was transferred to a 1.5 mL tube containing 1 mL of sterile water. The cells were completely suspended by vortex mixing for 30 s. In order to obtain bacterial cell pellets, the suspension was centrifuged at 10,000 rpm for 3 min and the supernatant was discarded. An aliquot of 100.0 µL of lysis buffer (40 mM Tris-acetate (pH 7.8), 20 mM sodium acetate, 1 mM EDTA and 1% SDS) was added to the bacterial pellet, followed by vigorous pipetting in order to lyse the cells. After lysis, 33.0 µL of 5M NaCl solution was added to the mixture. The contents of the tube were thoroughly mixed by inversion to remove most proteins and cell debris. The resulting viscous mixture was subjected to centrifugation at 12,000 rpm for 10 min at 4°C. After transferring the clear supernatant into another clean microcentrifuge tube, an equal volume of chloroform was added, and the tube was gently inverted at least 15 times until a milky solution was formed. The mixture was centrifuged at 12,000 rpm for 10 min and the aqueous phase containing the DNA was transferred to another clean microcentrifuge tube. Two equal volumes of chilled absolute ethanol were added to precipitate out DNA in the solution. The solution was mixed gently until a stringy white DNA precipitate was formed that condensed into a tight mass. The precipitate was incubated at -20°C in a freezer, overnight. Afterwards, centrifugation at 12,000 rpm for 10 min was done to obtain the pellet and the alcohol was discarded. The pellet was washed twice with 200.0 µL of 70% ethanol, air dried in the laminar flow hood for 30 min and then dissolved in 30.0 µL TE buffer (pH 8).

Both genomic DNA and pure colony cell suspension were used as template for PCR reactions to amplify 16S rDNA of the bacterial isolates. Around 10-20 ng of DNA was used as template in PCR reactions and around 10-20 cells/mL were used as template in cell suspension PCR. For the 25 uL PCR reaction mixture, the final concentration of PCR reagents (Invitrogen, MA, USA) was as follows: 1X PCR buffer without $MgCl_2$, 2.0 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 µM of forward primer and 0.5 µM reverse primer. *Taq* DNA polymerase was added at 0.04 U/µL to the DNA or cell suspension template at approximately 10-20 ng or 10-20 cells/mL per reaction, respectively. Universal 16S rDNA primers that were used in PCR are F8/rP2 (Amin *et al*., 2011) and 27F/1492Y (Thomas *et al*., 2007). The PCR program was set at 94ºC for 10 min for initial denaturation, followed by 32 cycles of 94ºC for 30 s, 55ºC for 40 s and 72ºC for 60 s, and final extension 72ºC for 5 min and indefinitely at 4ºC for storage. PCR amplicons were checked for correct size, at around 1300 bp and purity in 1% agarose gel stained with GelRed™ (Biotium, CA, USA). Amplicons were then sent for Sanger sequencing at AIT Biotech, Inc. (Singapore).

Bioinformatic Analysis and Identification of Bacterial Isolates using 16S rDNA

The bacterial endophytes and *Bacillus* spp. isolates were identified up to the closest species-level taxon using 16S rDNA gene sequence similarity to existing accessions in GenBank of the National Center for Biotechnology Information (NCBI). Sequences obtained using the two primer pairs F8/rP2 and 27F/1492Y were aligned first using the Basic Local Alignment Search Tool (BLAST) (Altschul *et al*., 1990) in NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The consensus sequence between the two amplicons underwent quality check and processingby trimming hanging and unaligned sequences deletion of gaps. After quality checking, the sequence became approximately 1300 bp, which then were used in succeeding analyses. BLAST was again used to search for sequences with high percent identity (85 to 100%) in NCBI GenBank.

Following BLAST analysis, the consensus sequences were subjected to phylogenetic analyses with the closest genera using Molecular Evolutionary Genetics Analysis software version 7 (MEGA7) (Kumar *et al*., 2016). Multiple sequence alignment following the ClustalW method was performed before inferring evolutionary tree using Neighbor-Joining method following the Tamura-Nei model. For comparison, sequence analyses were also done in EzBioCloud (www.ezbiocloud.net), a public data and analytics portal focusing on taxonomy, ecology, genomics, metagenomics, and microbiome of Bacteria and Archaea (Yoon *et al*., 2017; Kim *et al*., 2012). Several bioinformatics tools exist at EzBioCloud including those for nucleotide identity analysis (OrthoANI), pairwise alignment and editing (EzEditor) (Jeon *et al*., 2014; Lee *et al*., 2015) as well as for generating phylogenetic trees.

Antimicrobial Assay using Papaya Latex Water-soluble Fraction

Latex was harvested from large green fruits of the different papaya regrowth hybrid genotypes during early in the morning at around five to six o'clock. The fruits were swabbed with a cotton wool moistened with 70% ethanol before scarring. Sterile bamboo sticks with sharp and pointed tip were used to scar the fruit and collect the latex in a sterile 1.5-mL Eppendorf tube. The tubes were filled halfway with latex to leave some space for 500 µL sterile water. The latex was suspended in sterile water by vigorous flicking or pipetting. To separate the water fraction, the suspension was centrifuged at 3000 rpm for 10 min at 4ºC and was filter-sterilized using a 0.2 µm-pore size polyether sulfone (PES) membrane filter (Sterile Acrodisc, Gelman

Sciences or Whatman, GE Life Sciences). The water-soluble fraction was transferred to new 1.5-mL tubes and stored at freezing temperature (-10 or -20ºC) for future use.

A qualitative antimicrobial assay was developed in this study to test for inhibitory activity of papaya latex using bromcresol purple as indicator dye. A diluted suspension of the 72-h old culture (log phase) of *E. mallotivora* in PSA was prepared to obtain approximately 10^5 cfu/mL mL or OD_{600} at 0.45-0.50. On the other hand, diluted solutions of the stock water soluble fraction of the latex were prepared at 40, 65 and 80% v/v dilutions. The latex water-soluble fraction was added in a 1:1 proportion in a 1.5-mL tube and incubated at room temperature at varying time exposure (18, 30, 48 and 66 h). Stock suspension of *E. mallotivora* cells, sterile water and blank wells served as positive, negative and blank control setups, respectively. A 10 µL-aliquot of the solutions was inoculated in individual wells of a 96-well microtiter plate (Tarsons, Cat. No. 941146) that contains solidified PSA-bromcresol purple media. Bromcresol purple served as the indicator dye which turns yellow when there is a decrease in pH due to the active growth and acid formation of bacteria using the substrates in the medium.

The assay was terminated after two days of incubation when the change in color of each well was noted, from the original dark purple to bright yellow. Three replicates were prepared wherein four wells (samples) were allotted. Thus, for each treatment, a 4x12 matrix of wells was prepared. Color change assessment followed color change scoring of 1 (no change), 3 (changed to light purple), 5 (changed to light yellow), 7 (changed to yellow), 9 (changed to bright yellow). Using average scores, evaluation of antimicrobial activity was set as 7-9 (no significant antimicrobial activity), 3.1-6.9 (minimal antimicrobial activity) and 1-3 (with significant antimicrobial activity). The experiment was done twice to confirm results.

Antimicrobial Assay using Autoclaved Culture Metabolites (ACMs)

A suspension of the 72-h old culture (log phase) of the endophytic bacteria and *Bacillus* isolates in PSA was diluted to obtain approximately 10^8 cfu/mL or OD_{600} at 0.75-0.80. A 1.0 mL aliquot consisting of approximately 10^8 cells was inoculated in 100 mL of 3% tryptic soy broth (TSB, BD-Difco) in Erlenmeyer flasks. The broth cultures were incubated for 72 h at room temperature with continuous shaking at 120 rpm. Thereafter, the bacterial broth cultures were incubated for 30 min at autoclaving temperature (121ºC) and pressure (15 psi) in order to retain only the autoclaved culture metabolites (ACMs). The ACMs were centrifuged at 12,000 rpm for 10 min to separate cell debris. The supernatant was then filter-sterilized using a 0.2 µm-pore size polyether sulfone (PES) membrane filter (Sterile Acrodisc, Gelman Sciences or Whatman, GE Life Sciences) to remove remaining bacterial cell debris. The ACM filtrate was used in the succeeding assay for inhibition of *E. mallotivora.*

The ACMs were further diluted in order to obtain approximately 0.25 to 0.30 OD at 600 nm. Log phase colonies (72-h old) of *Erwinia mallotivora* was prepared as suspension in sterile water at 0.25 to 0.30 OD at 600 nm, in order to obtain approximately 10^4 cells/mL. The assay was done in a 96-well microtiter flat-bottom polycarbonate plate (Tarsons, Cat. No. 941146). ACMs and *E. mallotivora* cells were mixed in a well at 1:1 ratio (50 µL + 50 µL). Wells that served as controls contain *E. mallotivora* cells only (negative control), *E. mallotivora* cells in tryptic soy broth (negative control), tryptic soy broth only (blank) and sterile water only (blank). The OD_{600} reading and $\log_{10}$ cfu/mL of the wells at 0, 24, 48 and 72 h were noted and compared across treatments. In order to have a good estimate of the inhibitory effect of the ACMs, percent inhibition was calculated using the formula: $\frac{OD_{Em+TSB}-OD_{ACM}}{OD_{Em+TSB}} \times 100\%$, where

OD_{Em+TSB} is the optical density reading of the negative check (Em+TSB) at a given time point and OD_{ACM} is the optical density of any ACM-treated *Em* suspension at a given time point. This assay followed a randomized complete block design (RCBD), wherein the ACMs served as treatments and replicates as the blocking factor. Three replicates were prepared for each treatment containing three samples with 12 subsamples. Thus, for each treatment, 96 readings were done every time the OD values were assessed.

Preparation of Seeds and Plant Materials for Seed Defense Biopriming

The papaya seeds used in this study were obtained from the papaya germplasm collection at the Fruit Breeding Laboratory at Institute of Plant Breeding, College of Agriculture and Food Science, University of the Philippines Los Baños. The genotypes used were variety *Cariflora* (genotype 5648) which was evaluated as PRSV-tolerant and predominantly forming regrowth after crown rot infection and with good yield and fruit quality, and the *Solo* variety (genotype 501), which is susceptible to both PRSV and crown rot. Sterilized coir dust-garden soil medium was prepared as potting medium for the seeds.

ACMs were prepared as previously described above. Consequently, seeds were surface sterilized using 70% ethanol solution for 1 min, followed by rinsing twice in sterile water for 2 min and blot- and air-drying under the laminar flow hood for 15 min. The seeds were immersed in the ACMs for 16 h, followed by blot- and air-drying under the laminar flow hood for 30 min. Soaking in sterile tryptic soy broth only served as the mock treatment. The seeds were sowed in sterile potting medium with complete fertilizer and maintained under greenhouse conditions with daily watering for six weeks. The plants were ready for inoculation if it has 4-5 leaf nodes already and about 10 cm in height.

Germination and Growth Assessment after ACM Treatment

The effects of ACM treatment on seed germination, shoot and root length and biomass were assessed. Percent successful germination of seeds treated with ACMs were assessed after four weeks. Shoot and root length were measured after seven weeks. To evaluate the effect of ACM treatment on plant biomass, the 7-wk old plant samples were dried in an oven set at 65ºC. The dry weight of the samples were assessed after 36 h. This experiment followed a randomized complete block design (RCBD), wherein the ACMs served as treatments and replicates as the blocking factor. Separate statistical analyses were done for the two genotypes.

Inoculation of ACM-treated Plants and Disease Assessment

Six-week old ACM-treated seedlings of both papaya genotype 5648 and 501 were inoculated with *Erwinia mallotivora* as described by Supian *et al*. (2017) with some modifications. Pathogen challenge was done to test for the possible defense biopriming activity of the ACMs. The plants were watered first before inoculation to increase its internal turgor pressure and activate transpiration and water translocation, thus increasing inoculation efficiency. To further increase chances of infection, the apical tips, petiole and midrib of the second and third youngest leaf were pricked with a sterile syringe needle. On the other hand, the inoculum was prepared by suspending a 72-h old pure culture of *E. mallotivora* in sterile water and standardizing the suspension at OD_{600} of 0.75-0.80 to obtain approximately 10^8 cfu/mL of inoculum. The inoculum was applied through spraying three times per plant, delivering approximately 1.0 mL of inoculum. After inoculation, plants were covered with clear plastic cups in order to maintain high relative humidity and favor bacterial growth for 48 h.

Disease assessment was done at 7 and 21 days post inoculation (dpi). Percent disease incidence was calculated as PDI = $\frac{\text{no.of diseased plants}}{\text{total no.of plants}} \times 100\%$. Disease severity assessment followed the scoring used by Supian *et al*. (2017) with some modifications. This modified scale consisted of the following: 0 (no symptom), 1 (lesions are restricted to inoculation point only), 3 (lesions are expanding to less than half of leaf area), 5 (lesions are expanding to more than half of leaf area), 7 (lesions and rotting spread to the petiole and stem and 9 = (plant suffers severe rotting, crown falls off). Finally, percent disease severity index was calculated as (PDSI = $\frac{\text{total of severity scores}}{\text{total no.of plants} \times \text{max.severity score}} \times 100\%$. This assay followed a randomized complete block design (RCBD), wherein the ACMs served as treatments and replicates as the blocking factor. Three replicates were prepared for each treatment containing three samples with five subsamples each. Thus, for each treatment, 45 plants were allotted.

Statistical Analysis of Data

All *in vitro* experiments were done twice and greenhouse experiment were done thrice to confirm results. All data generated were subjected to statistical analysis through one-way analysis of variance (ANOVA at $\alpha = 0.05$) in SAS software program (SAS Institute, Inc.). Separate statistical analyses were done for the two papaya genotype used . The means were subjected to pairwise mean comparison using Tukey's honest significant difference (HSD) test. All tests are considered significant if $P < 0.05$ (*), $P < 0.01$ (**) or $P < 0.001$ (***).

Results

Papaya tissues harbor a bacterial community, after infection and prior to regrowth.

Interestingly, a community of bacterial cells, possibly of different genus or species was observed in the tolerant genotype, 5746 (var. *Cariflora*) in early stages of regrowth (3-4 wks), but not in fully regrowth tissues (7-8 wks). Fig. 1a,b shows the micrographs of healthy tissues, while Fig. 1c,d and Fig. 1e,f show the micrographs of the early and fully regrowth tissues, respectively. Moreover, in infeced tissues (Fig. 1g,h), rod-shaped cells, presumably *E. mallotivora* (*Em*), were observed to predominantly colonize open wounds of the papaya stem indicating that it is a probable mode of entry. The tissues shown in the micrograph were directly obtained from the water-soaked lesion in the stem, which is one of the typical symptoms of the bacterial crown rot disease. Moreover, cuticular ridges of the papaya stem were also commonly found to harbor *Em* cells, while forming biofilms, probably through exopolysaccharide production (Fig. 1i,j).

Bacteria from regrowth plants have distinct cultural and biochemical properties.

Building on the SEM observations, cultural detection was done to isolate regrowth-associated viable bacteria. The regrowth stem and roots, were surfaced sterilized prior to ooze out test, thus eliminating the presence of epiphytic microorganisms. Two culturally distinct endophytic bacteria (EBW, EBY) and three *Bacillus* (BN, BS, BT) species were isolated in regrowth stems and roots, respectively. Based on *Bergey's* Manual of Systematic Bacteriology (Brenner *et al.*, 2009), EBW was identified under genus *Kosakonia* (Gammaproteobacteria: Enterobacterales: *Erwiniaceae*), while EBY was identified under genus *Sphingomonas* (Alphaproteobacteria: Sphingomonadales: *Sphingomonadaceae*). On the other hand, the *Bacillus* isolates were not

398 easily identified using phenotypic charecteristics because their colonies are very much alike
399 and also exhibit similar biochemical properties. The *Bacillus* isolates are gram-positive, rod-
400 shaped and spore-forming that forms irregular, undulate colonies with dry consistency in
401 PDPA. Moreover, all the isolates were not able to infect papaya nor cause any hypersensitive
402 reaction, which implies no interaction with plant resistance machinery. The summary of
403 characteristics of the isolates is shown in Table 1.
404
405 **Phylogenetic analysis revealed diverse taxon of bacterial isolates.**
406
407 Identification using 16S rDNA sequence was carried out to determine the most probable taxa
408 of the bacterial isolates. Initially, the sequences were trimmed in MEGA7 by retaining high-
409 peak signals from the Sanger sequence file. Thereafter, the consensus sequence of the two
410 amplicons of the two primers were obtained after alignment using ClustalW in MEGA7. The
411 consensus sequences were used in BLAST in NCBI-GenBank (Altschul *et al.,* 1990) and
412 further analysis in EzBioCloud-Identify (Yoon *et al.,* 2017). EzBioCloud served as
413 confirmation of BLAST results because it contains valid names or type specimens. On the other
414 hand, phylogenetic analysis revealed the most probable taxon and species-level identity of the
415 isolates. Fig. 2 shows the resulting phylogenetic trees with the BLAST percent identity to valid
416 taxa identified in EzBioCloud. The trees were constructed using the Neighbor-Joining method
417 (Saitou and Nei, 1987) and following the Tamura-Nei model (Tamura and Nei, 1983), where
418 boostrap analysis was conducted using 1000 replicates (Felsenstein, 1985).
419
420 The analyses showed that EBW is paraphyletically related to *Kosakonia* sp. clade and
421 monophyletically related the *Enterobacter* sp. clade. *Kosakonia* sp. is a new genus of plant-
422 associated *Enterobacter* species. The bootstrap value (BV) of EBW with *Kosakonia* sp., clade
423 is very low (<50%), which indicates that it could be a new species of *Kosakonia*. Moreover,
424 EBY is paraphyletically related to *Sphingomonas phyllosphaerae* FA2 (Rivas *et al*. 2004) and
425 *S. endophytica* YIM 65583 (Huang *et al.,* 2012) (BV=0.91). On the other hand, isolates BN
426 and BS were identified to belong to the *B. amyloliqufaciens* clade, specifically BS forming a
427 monophyletic group with *B. amyloliquefaciens*, but with low BV and BN being
428 paraphyletically related to the clade at BV=0.96. On the other hand, isolate BT belongs to the
429 *B. thuringiensis* clade and has a paraphyletic relationship (BV=0.49). BVs below 50%, were
430 considered insignificant, thus these isolates might need to undergo further phylogenetic
431 analysis using other gene sequences.
432
433 ***E. mallotivora* was promptly and efficiently inhibited by papaya latex and Autoclaved**
434 **Culture Metabolites (ACMs).**
435
436 No study was conducted yet that tested the antimicrobial effect of the latex extract on the
437 growth of *E. mallotivora* (*Em*), *in vitro*. The assay used in this study was successful in
438 qualitatively showing *Em* inhibition by exposure to latex water-soluble extract and was first of
439 its kind and originally developed in this study. Fig. 2 shows that there is no inhibition of
440 bacterial growth at 18 h in any concentration, except for 100% concentration wherein there is
441 only slight inhibition. Partial bacterial inhibition was observed starting 30 h of exposure while
442 complete inhibition was observed at 48-66 h with 50% water-soluble latex concentration.
443
444 The microtiter plate assay for the inhibition of *Em* was proven to be useful in evaluating
445 inhibition efficacy of the autoclaved culture metabolites (ACMs) by just monitoring the
446 absorbance readings. Fig. 4 shows the trend in absorbance and estimated bacterial population
447 ($\log_{10}$ cfu/mL) of the ACM treatments, as well as percent relative inhibition. After 24 h, a

448 drastic increase in cell density was observed in the BS, BT and EBY treatments. Nevertheless,
449 this observed increase in cell density in the ACM treatments was still considerably lower than
450 the negative control, at around 2-3 folds. Thus, there is still a significant inhibition of cell
451 multiplication in these treatments. More importantly, bacterial growth was highly inhibited
452 when *Em* was subjected to BN and EBW at 60-65% and 53%, respectively.
453
454 **All ACMs reduced growth vigor of Papaya, except ACM from *Kosakonia*.**
455
456 Treatment of surface-sterilized papaya seeds with ACMs from bacterial endophytes and
457 *Bacillus* isolates resulted to retarded germination compared to mock-treated seeds. Fig. 5a
458 shows the average percent germination of papaya seeds of genotype 501 (var. *Solo*) and 5648
459 (var. *Cariflora*), which were evaluated as susceptible and tolerant (*i.e.* regrowth) to the crown
460 rot disease, respectively. Among the all treatments, BS has no significant adverse effect on the
461 germination of papaya seeds in both genotypes. However, treatment with isolate BN and BT
462 resulted to a significant reduction in percent germination of 501, while treatment with isolate
463 EBW and EBY resulted to lowest percent germination in genotype 5648. This implies that
464 papaya might respond differently to ACM treatments, depending on genotype.
465
466 Plant growth was assessed by getting the dry biomass of plants at 7 wks after sowing. Fig. 5b
467 shows the accumulated biomass, measured as oven-dried weight, of the ACM-treated plants.
468 Treatment with ACM from EBW yielded the highest dry weight, particularly in genotype 5648,
469 which correspond to more than two-fold increase relative to mock. However, no significant
470 increase in biomass was observed in genotype 501 in the same treatment, indicating that growth
471 stimulation by bacterial ACMs in papaya might be genotype-dependent. All other treatments
472 significantly reduced biomass accumulation in both genotypes as depicted, especially in BS
473 ACM-treatment. Finally, Fig. 5c visualizes the combined relative effect of the ACM
474 treatments, wherein EBW clearly showed a general positive positive effect the germination and
475 biomass of the two genotypes.
476
477 **ACMs significantly reduced the incidence and severity of BCR disease in Papaya.**
478
479 The possibility of using easy to prepare, sterile, highly-stable, and potent metabolites from
480 beneficial bacteria as disease resistance bioprimers in papaya was assessed in this study.
481 Although plant vigor was adversely affected by the treatment of ACMs, the papaya plant would
482 eventually recover and grow normally. Fig. 6 shows the representative photographs of
483 inoculated plants after two weeks of observation. Based on observations made, BN-treated
484 plants in both genotypes seemed to have retarded growth after 14 dpi, due to infection. Necrosis
485 within half of the inoculated leaf area was commonly observed in these plants. BS- and BT-
486 treated plants in both genotypes were not significantly affected by the pathogen challenge and
487 continued to grow normally. At most instances, plants in this treatment only had necrosis at the
488 inoculation point. Recorded percent disease incidence (Fig. 7a) revealed that in general, ACM-
489 treated plants have lower propensity to bacterial crown rot infection. BCR incidence continued
490 to increase until 21 dpi, but the incidence in ACM-treated plants remained lower compared to
491 that of the mock-treated plants, in both genotypes. On the other hand, there was a general
492 reduction in average severity ratings in both genotypes (Fig. 7b). More importantly, a two-fold
493 reduction in disease severity was observed in EBW treatment at 21 dpi relative to control.
494 Lowest severity was observed in EBW-treated 5648 plants relative to control. Generally,
495 improved resistance is more pronounced in 5648 (resistant) plants than in 501 (susceptible)
496 plants as visualized in Fig. 7c.
497

Discussion

BCR tolerant genotypes such as 5648 (var. *Cariflora*) were primarily identified using the lateral stem 'regrowth' phenotype observed at 5-7 wks after infection (wai). In this study, it was shown through SEM observations that infection sites in 5648 were colonized by a community of bacteria at the onset of regrowth (3-4 wai). However, these bacterial community was not observed later at 5-7 wai, when there is full stem regrowth already. Other observed innate phenomena such as latex production and tissue hardening (*i.e.* lignin deposition) might improved and hastened the recovery from the disease. Moreover, this recovery was shown to be promptly and efficiently aided by the papaya latex as it inhibited *E. mallotivora* proliferation *in vitro*. Due to the significant role of latex, it was also hypothesized that latex-resistant microbes inhabiting the plant vascular bundles, might also contributed to disease tolerance.

The importance of the microbiome in relation to tolerance or resistance against bacterial disease in plants was recognized as early as the 1960's. Phytopathogenic bacteria, particularly of genus *Erwinia,* remain as epiphytes in a so-called 'resident phase' on the surface of their hosts. Proliferation of epiphytic microflora, including the pathogenic ones, is then triggered by favorable environmental conditions such as high relative humidity and moderate to warm temperatures (Leben, 1965). In the present study, epiphytic bacteria which include *Erwinia* species, were not observed in the surface of the regrowth tissues, which probably indicated that these bacteria returned to its 'viable but not culturable' (VBNC) and harmless state during the recovery of the plant. Interestingly, the VNBC state was observed in *Ralstonia solanacearum* and *Erwinia amylovora* as its over-seasoning and survival strategy, respectively (Grey and Steck, 2001; Ordax *et al*., 2006). Similarly, reoccurrence of crown rot disease in regrowth plants was reported (Magdalita *et al*., 2016), suggesting the possibility of VNBC state of *E. mallotivora*. Collectively, these observations might indicate that the papaya plant, through the help of beneficial microbes and latex was able to tolerate bacterial infection and continue growth by forming lateral stem regrowth.

Building around the initial observations, three rhizoplane and two endophytic bacteria were isolated from regrowth genotype 5648, and further characterization and identification using 16S rDNA were carried out thereafter. Root-associated *Bacillus* species were identified under the *B. amyloliquefaciens* clade (isolate BN and BS) and one under the *B. thuringiensis* clade (isolate BT), while the two endophytic bacteria were identified under genus *Kosakonia* (isolate EBW) and *Sphingomonas* (isolate EBY). *Bacillus velezensis* strain CR-502 (isolate BN) was known to produce surfactants that may confer antimicrobial activity (Ruiz-Garcia *et al.,* 2005). Additionally, antibiotics from *Bacillus* successfully inhibited gram-positive bacteria that are also human-pathogenic such as *Stapylococcus aureus, Clostridium difficile* and *Listeria monocytogenes* (Collins *et al.,* 2016). This study is the first instance that *B. velezensis* was shown to have inhibitory properties against a bacterial plant pathogen.

Species of the genus *Sphingomonas* sp. are reported to have beneficial properties and are usually associated as endophytes in many plants. In particular, *S. paucimobilis* associated with an orchid plant (*Dendrobium officinale*), was proven to have growth promoting properties by increasing phytohormone production and aiding in atmospheric nitrogen fixation, processes which the plant cannot carry out normally (Yang *et al*., 2014). Likewise, *S. phyllosphaerae* and *S. endophytica* were originally isolated as endophytes in *Acacia caven* and *Artemesia annua*, respectively. On the other hand, *Kosakonia* sp. (formerly *Enterobacter* sp.) is a new taxon name assigned to endophytic, plant-associated Enterobacteraceae species. Two species, *K. oryzophilus* and *K. oryzendophytica*, were found to be key inhabitants of rice root endosphere

548 (Hardoim *et al.,* 2013). The two species were able to promote rice growth by supplying nitrogen
549 and phosphorus to the plant. Similarly, *Kosakonia sacchari* (Zhu *et al.,* 2013) (formerly
550 *Enterobacter* sp.) is an endophyte associated with sugarcane stem, roots and rhizosphere. The
551 bacterium was shown to help the plant in producing fixed nitrogen for its consumption (Zhu *et*
552 *al.,* 2013). Another species of *Kosakonia,* named *K. pseudosacchari,* was discovered to be
553 endophytically associated with field grown corn (*Zea mays*) root tissues. *K. pseudosacchari* is
554 also a closely related species of *K. sachari* and *K. oryzedophytica* (Kampfer *et al.,* 2016).
555 However, there were no studies were reported yet regarding the antimicrobial properties of
556 *Kosakonia* and *Sphingomonas* species other than this study. Interestingly, the aforementioned
557 *Bacillus* species and endophytic bacteria were not able to infect papaya plants nor cause any
558 hypersensitive reaction and is resistant to latex exposure (data not shown). A similar et of
559 endophytic bacteria were also isolated from and constantly associated with tissue-cultured
560 papaya plants based on previous studies (Thomas and Kumari, 2010; Thomas *et al*., 2007a;
561 Thomas *et al*., 2007b).
562
563 Endophytic bacteria and various *Bacillus* species associated with plants were reported to have
564 growth-promoting and disease resistance-inducing properties (Hallmann *et al.,* 1997; Ongena
565 and Jacques, 2007; Podolich *et al.,* 2015). Species of *Bacillus*, such as *B. paralicheniformis*
566 MDJK30 was reported to promote growth in *Arabidopsis thaliana* (Dunlap *et al.,* 2015;
567 Palacio-Rodriguez *et al.,* 2017) and suppress growth of *Fusarium solani* (Wang *et al*., 2017).
568 Another strain, *B. paralicheniformis* KMS 80 was discovered to be associated with rice root
569 endosphere and helped the plant in nitrogen fixation (Annapurna *et al.,* 2018). However, assays
570 for growth-promotion and disease resistance induction heavily rely on the availability of
571 culturable isolates and its compatibility or ability to colonize the host plant. In this study, these
572 challenges were surpassed by using culture metabolites that are easy-to-prepare, sterile and
573 highly stable, thus the term, autoclaved culture metabolites (ACMs). Moreover, seed defense
574 biopriming using ACMs of bacteria is a relatively new method used in inducing systemic
575 disease resistance in plants. This method was previously demonstrated in pepper and cucumber
576 using ACMs of *Bacillus gaemokensis* PB69 (Song *et al.,* 2017). The presence of potent
577 metabolites in high and unstandardized concentrations in the ACMs might have caused the
578 reduced germination and vigor in papaya seeds. On the other hand, treatment of live cell
579 suspensions of endophytic and other beneficial bacteria also led to reduced germination and
580 seedling vigor in papaya (Thomas *et al.,* 2007), tomato (Worrall *et al.,* 2012) and oil palm (Azri
581 *et al.,* 2018), but recovery of plant growth was observed when grown in greenhouse conditions.
582 The inoculated plants were able to grow normally and were able to accumulate more biomass
583 compared to the normal or untreated ones.
584
585 Moreover, treatment of *Sphingomonas paucimobilis* live cells in *Dendrobium officinale*
586 resulted to the increase in plant growth due to increased phytohormone production and nitrogen
587 fixation (Yang *et al.,* 2014). In oil palm, biofilm production is reported to be the mechanism
588 that rendered growth promoting ability of a *Bacillus salmalaya* isolated from agricultural soils
589 in Malaysia (Azri *et al.,* 2018). Inoculation of the bacterium resulted to longer shoot and root
590 growth as well as higher biomass accumulation in oil palm seedlings. These reports are clear
591 indications of the involvement of plant-associated bacteria in growth promotion in plants.
592 However, there are no extensive studies yet on the effects of ACMs on plant growth, aside
593 from the report by Song *et al.* in 2017. Their team reported that ACMs from *B. gaemokensis*
594 PB69 stimulated defense responses and reduced *Pseudomonas* infection in pepper and
595 cucumber from seed to seedling stage, without significantly reducing plant growth and
596 biomass. In papaya, the effect of endophytic bacterial isolates on the growth of
597 micropropagated plants was also investigated. Relative to the control plants, treatment with the

live endophytic bacteria *Microbacterium* and *Sphingomonas* resulted to an increase in root and shoot biomass (Thomas *et al.,* 2007a). Similarly, treatment of *Bacillus gaemokensis* PB69 ACM in cucumber resulted to an increase in shoot biomass and fruit weight relative to the mock-treated plants (Song *et al.,* 2017). Results of the present study and that of the aforementioned studies clearly indicated the probable role of live bacterial cells and its metabolites in stimulation of growth and biomass accumulation in plants.

On the other hand, ACMs from the endophytes contributed to the reduction of the incidence of crown rot, in both susceptible and resistant genotypes. The plants remained healthy wherein only necrosis at the inoculation site was observed in most of the samples, indicating reduced susceptibility to bacterial crown rot infection. These observations indicated that ACM-treatment has a potential to decrease the predisposition of the papaya plant to the crown rot disease and stimulate plant growth. Further analysis of percent disease severity index for each treatment led to the conclusion that, ACM-treated plants have significantly lower disease severity, relative to mock-treated plants. Interestingly, genotype dependence of papaya responses to ACM treatments might be important to consider in breeding programs to augment disease resistance and increase its durability. If optimally done, disease protection and induction of systemic resistance using beneficial microbes or its metabolites will be a good alternative to chemical pesticides or resistance inducers. Endophytic bacteria, actinomycetes and many *Bacillus* species were proved to have such bioactivity in plants, may it be its native host or a non-host (Tonelli *et al.,* 2011; Yang *et al.,* 2014; Wicaksono *et al.,* 2018; Rajamanickam *et al.,* 2018). However, induction of defense responses in plants comes with a penalty or cost (Cipollini *et al.,* 2003; Dietrich *et al.,* 2005) by delaying and lowering the plant's germination and seedling vigor. However, the fully-grown seedlings eventually recuperate and even accumulate higher biomass relative to the untreated counterpart.

On the other hand, the use of autoclaved culture metabolites (ACMs) is relatively new and only a few studies were reported to prove resistance-inducing bioactivity in plants. The use of ACMs provides a convenient way to prime defense in seeds because this method is easy to prepare, and the metabolites produced are highly stable and potent. Many reports indicated that cyclic dipeptides from *Bacillus* species have antimicrobial and disease inducing properties (Song *et al.,* 2017; Liu *et al.,* 2017). *Pseudomonas* infection in *Arabidopsis* was reduced upon application of cyclic dipeptides produced by *Bacillus vallismortis* BS07 (Noh *et al.,* 2017). Among the identified compounds cyclo(L-Ala-L-Leu) were the most effective in reducing pathogen multiplication *in planta.* Similar observations were made in cucumber and pepper where cyclo(Leu-Pro) were the most effective in reducing disease severity and pathogen population *in planta* (Song *et al.,* 2017). However, the use of live cell suspension remains the most popular method in exploiting beneficial microbes to promote better plant health (Hallmann *et al.,* 1997). Thus, comparative studies on the effect of live bacterial cell or ACM treatment on plant germination and growth might be a good topic for future investigations.

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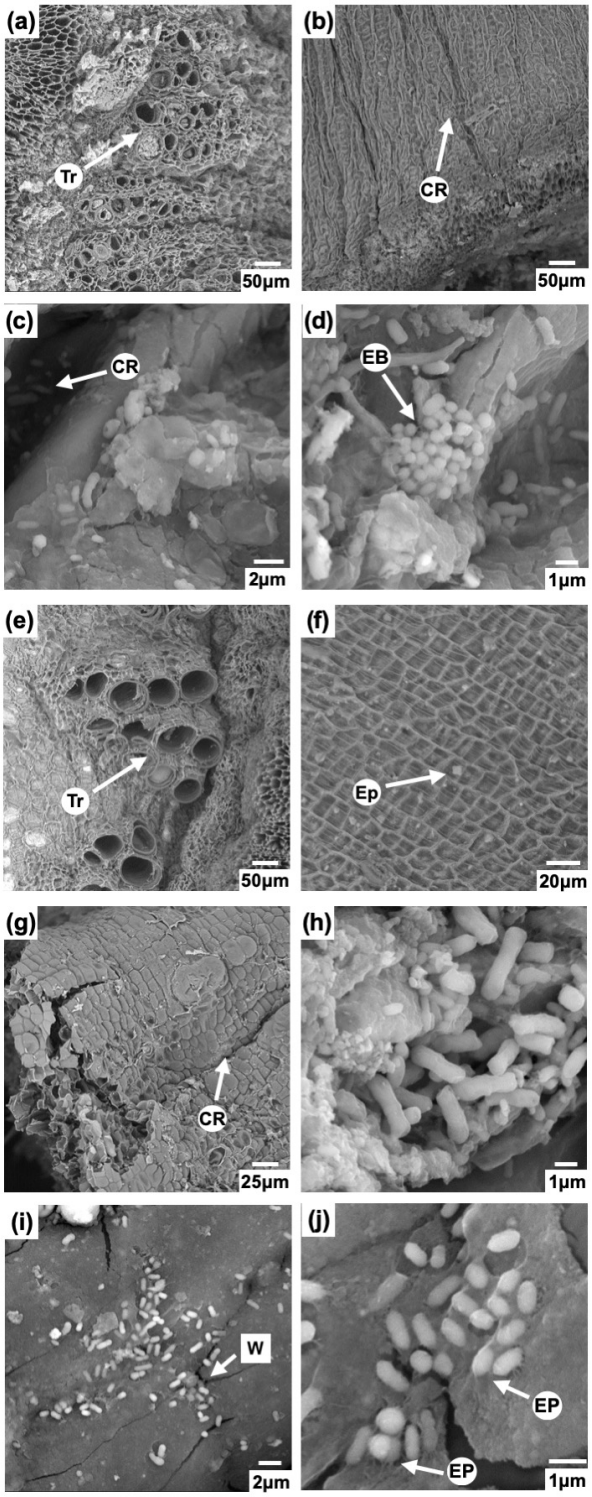

Figure 1. Scanning electron micrographs showing bacterial communities associated with the regrowth genotype 5648 (var. *Cariflora*). (a, b) healthy tissues from 5648; (c, d) early regrowth tissues from 5648; (e, f) full regrowth tissues from 5648; (g, h) infected tissues from 501 genotype (var. *Solo*) and; (i, j) biofilm production and dwelling on cuticular ridges by bacterial communities in 5648. Note: Tr (tracheids), CR (cuticular ridge), Ep (epidermis), CR (cuticular ridge), W (wound), EP (exopolysaccharide), EB (other epiphytic bacteria). Scale bars are shown in each micrograph.

815 Table 1. Summary of morpho-cultural and biochemical properties of the bacterial isolates.

Properties	*E. mallotivora*	*Kosakonia* sp.	*Sphingomonas* sp.	*Bacillus* sp. BN	*Bacillus* sp. BS	*Bacillus* sp. BT
Cell / Single Colony[1]						
colony color	cream white	white	deep yellow	light brown	cream white	cream white
cell shape	rods	rods	short rods	rod	rod	rod
gram stain	negative	positive	negative	positive	positive	positive
spore formation	absent	present	absent	present	present	present
diameter (mm)	0.8-1.0	6.0-6.5	5.5-6.0	6.0-6.5	7.0-7.5	5.0-6.0
shape	circular	circular	circular	irregular	irregular	irregular
margin	entire	entire	entire	undulate	undulate	undulate
elevation	convex	convex	convex	umbonate	flat	raised
consistency	fluidal	fluidal	butterous	dry	dry	dry
Morpho-cultural[2]						
nutrient agar stroke	filiform, butyrous	filiform, viscid	filiform, butyrous	effuse, butyrous	effuse, rubbery	rhizoid, brittle
nutrient broth	flocculent	ring	flocculent	membranous	membranous	membranous
Biochemical[3]						
oxygen requirement	facultative anaerobe	facultative anaerobe	facultative anaerobe	obligate aerobe	obligate aerobe	obligate aerobe
catalase	+	+	+	+	+	+
cysteine desulfurase	-	+	+	+	-	-
lipase	-	+	+	+	+	+
gelatinase	-	+	-	+	+	+
starch utilization	+	-	+/-	+	+	-
cellobiose utilization	-	+	+	+	+	+
glucose utilization	+	+	+	+	+	+
lactose utilization	-	+	+	+	+	+
maltose utilization	-	+	+	+	+	+
mannitol utilization	+	+	-	+	+	+
mannose utilization	+	+	+	-	-	-
sorbitol utilization	-	+	-	+	+	+

816 [1]Colony morphology in Peptone Sucrose Agar (PSA) after 7 d of room temperature incubation.
817 [2]Cultural properties after 14 d of room temperature incubation.
818 [3]+, positive test; -, negative test; +/-, weak positive.

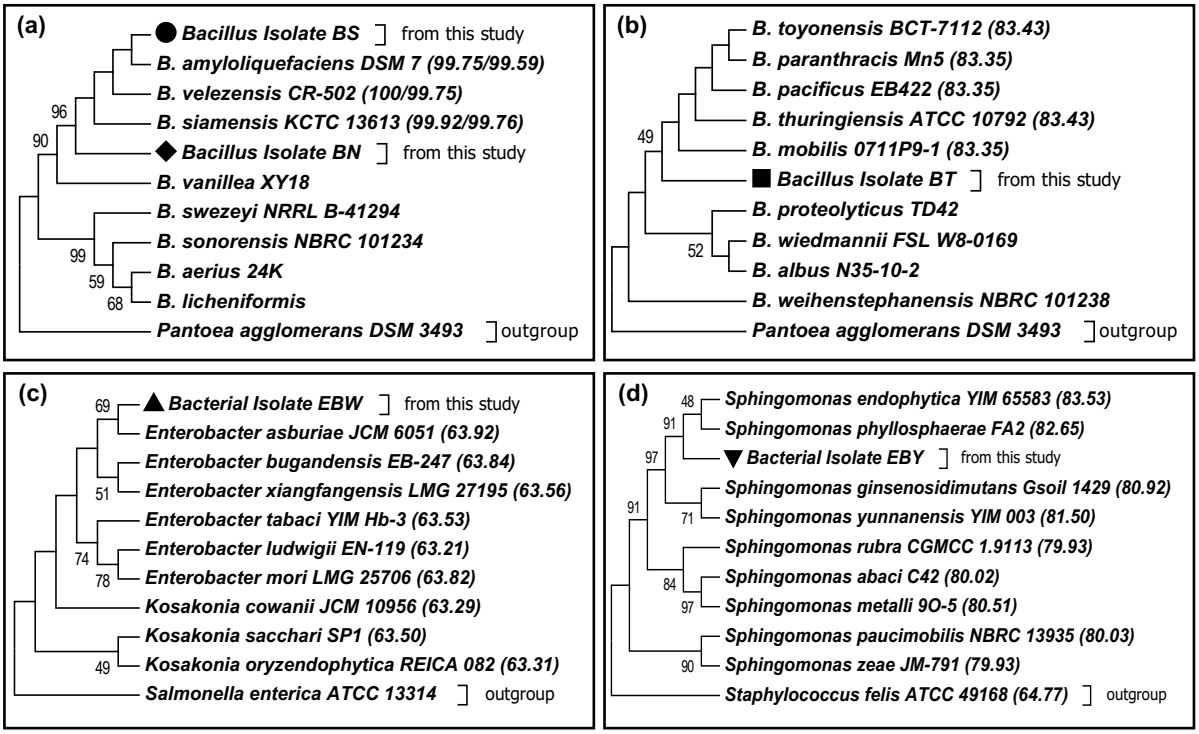

Figure 2. Phylogenetic trees showing most probable taxa and species-level identity based on 16S rDNA sequences of: (a) Isolate BN and BS tree showing relationship to isolates in *B. amyloliqufaciens* clade. percent identity values to BN/BS are shown in members of the clade; (b) Isolate BT tree showing relationship with *B. thuringiensis* clade, percent identity to isolate BT are shown in members of the clade; (c) Isolate EBW tree showing relationship with *Enterobacter/Kosakonia* clade, percent identities to EBW are shown in the members of the clade; (d) Isolae EBY tree showing relationship to the *Sphingomonas* clade, percent identities to EBY are shown in the members of the clade. The analysis followed Neighbor Joining following the Tamura-Nei model in MEGA7. Bootstrap values for 1000 replicates greater than 45% are shown in the nodes.

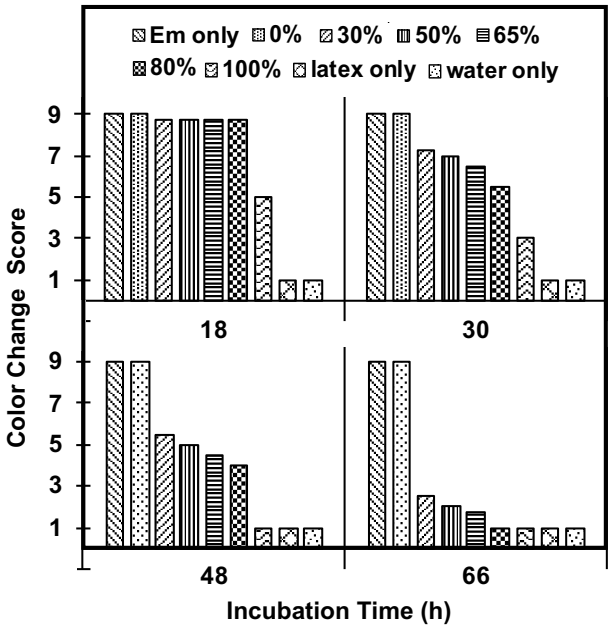

Figure 3. Antimicrobial effect of papaya latex on *Erwinia mallotivora.* Average color change scores of the latex inhibition assay, showing the treatments at different incubation time and latex concentration. Data were taken from the average of two trials. Evaluation of color change scores are as follows: 7-9 (no significant antimicrobial activity), 3.1-6.9 (minimal antimicrobial activity) and 1-3 (with significant antimicrobial activity).

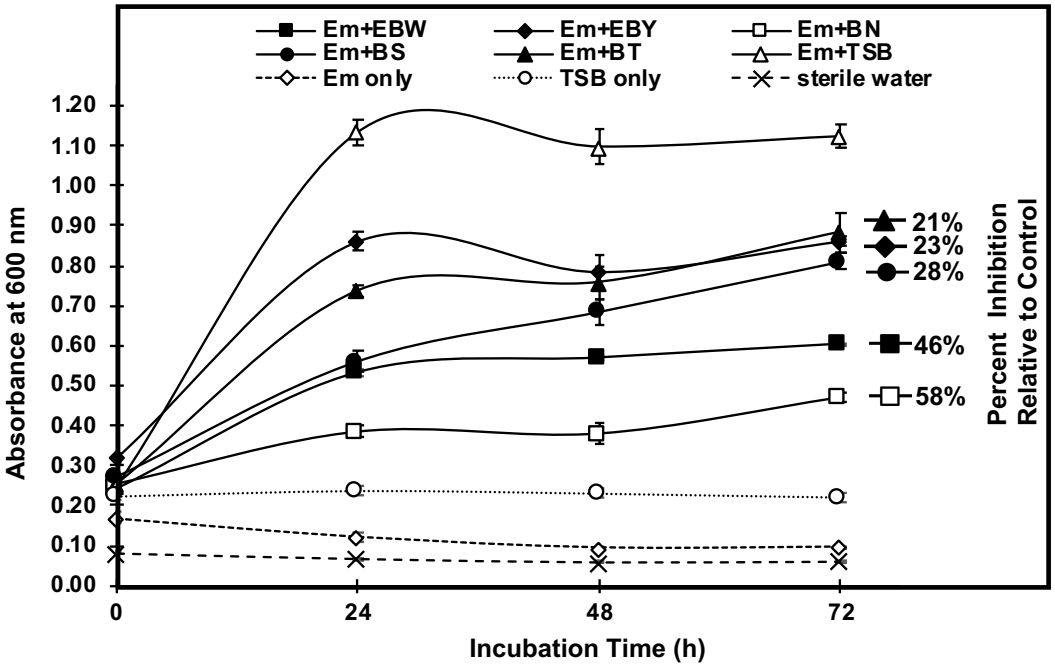

Figure 4. Antimicrobial effect of autoclaved culture metabolites (ACMs) on *Erwinia mallotivora.* Changes in absorbance readings (left axis) and percent inhibition (right axis) of the autoclaved culture metabolite-treated *Erwinia mallotivora* (Em) cell suspensions are shown. The treatments are as follows: Em treated with ACM from isolate EBW (*Kosakonia* sp.) (Em+EBW), EBY (*Sphingomonas* sp.) (Em+EBY), isolate BN (Em+BN), isolate BS (Em+BS) and isolate BT (Em+BT). Em in tryptic soy broth (Em+TSB) and Em only served as negative controls while TSB only and sterile water only served as blanks.

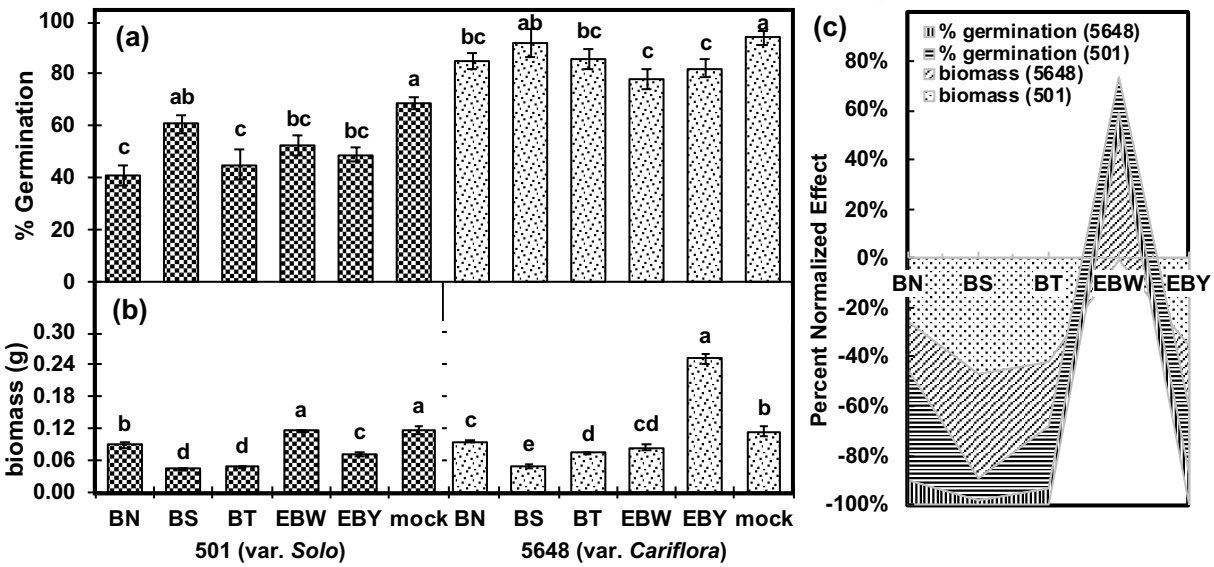

Figure 5. Effect of autoclaved culture metabolites (ACMs) on the growth vigor of papaya. (a) Germination data was taken 21 days after sowing (das), while (b) biomass was taken 49 das, (c) percent normalized effect calculated relative to the mock treated seedlings.

Figure 6. Representative photos of pathogen-challenged ACM-treated plants of (a) genotype 501 and (b) genotype 5648. Scale bars are shown in the figure above.

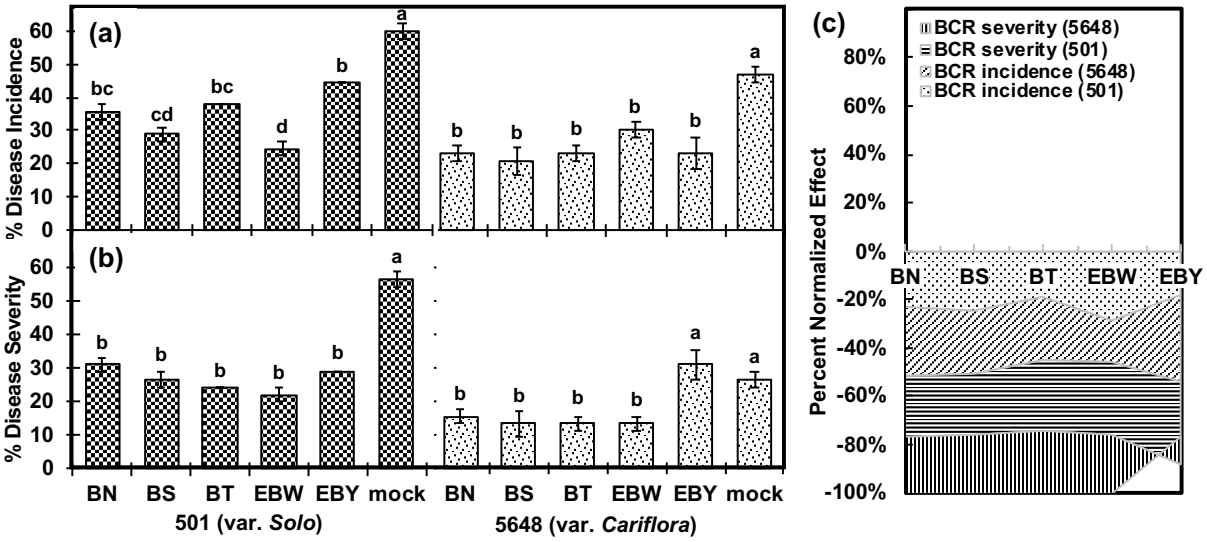

Figure 7. Effect of autoclaved culture metabolites (ACMs) on the incidence and severity of bacterial crown rot disease of papaya. (a) average percent disease incidence taken at 21 days after inoculation (dai), (b) average percent disease severity at 21 dai, (c) percent normalized effect calculated relative to the mock treated seedlings.