



Palm oil decanter cake wastes as alternative nutrient sources for production of enzymes from *Streptomyces philanthi* RM-1-138 and the efficacy of its culture filtrate as an antimicrobial agent against plant pathogenic fungi and bacteria

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Abstract

Valorization of solid wastes from palm oil mill based medium for the production of enzyme from *Streptomyces philanthi* RM-1-138 and the efficacy of its culture filtrate as an antimicrobial agent against plant pathogenic fungi and bacteria were investigated. Solid-state fermentation of *S. philanthi* RM-1-138 grown in oil palm decanter cake (OPDC), palm pressed fiber (PPF), and OPDC + PPF (1:1) for production of enzymes was investigated after 12 days incubation. OPDC was the best substrate as it gave the highest enzyme activity of xylanase, cellulase, and chitinase (at 5.21 ± 0.26 , 3.75 ± 0.38 , and 0.48 ± 0.01 U mL⁻¹ respectively) at 10 days incubation. In addition, the production of β -1,3-glucanase was not detected in all raw materials. From our previous studies, the culture filtrate of this strain was found to contain bioactive compounds. In this study, the efficacy of the culture filtrate RM-1-138 produced under the optimal conditions (60% moisture content with the initial pH at 7.0) was evaluated against three strains each of plant pathogenic fungi and bacteria. Its efficacy was most pronounced in *Ganoderma boninense* (74.33%) followed by *Curvularia oryzae* (64.67%) and *Ceratocystis paradoxa* TT1 (58.33%), respectively. Its efficacy against *Xanthomonas axonopodis* pv. *glycines*, *X. oryzae* pv. *oryzae*, and *X. campestris* pv. *campestris* were low and not significantly different ($P > 0.05$) (19.17, 18.50, and 18.33 mm, respectively). With these results, the OPDC had a high potential to be utilized as substrate for the production of enzymes and antimicrobial agent by *S. philanthi* RM-1-138.

Keywords Decanter cake · Palm pressed fiber · Solid-state fermentation · Bioactive compounds · Enzyme · *S. philanthi* RM-1-138

1 Introduction

Palm oil is one of the five major edible oils in the world. In Thailand, the production of palm oil has become one of the most important agro-industries during the last 20 years,

mainly in the eastern and southern regions of the country [1, 2]. A typical palm oil mill generates a substantial mass of wastes for every ton of fresh fruit bunches; empty fruit bunches (20–28%), oil palm decanter cake (OPDC) (4%), and palm pressed fiber (PPF) (11–12%) [3]. OPDC is the solid waste generated after processing the oil sludge through the decanter (or 3-phase separator) while PPF is generated after passing the digested oil palm fruits through double screw press, air classifier, and fiber separator, respectively. Basically, the OPDC still contains 30–40% of residual and is currently disposed of directly in landfills without treatment [4], causing severe water and air pollution with a high environmental impact on the sustainability of the palm oil industry.

A current problem in the south of Thailand is to manage the wastes generated during palm oil milling processes.

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OPDC has high organic carbon (85.9%, volatile solid) and a nutrient-rich composition (2.9%, total Kjeldahl nitrogen) content [5] in the form of oil and fibrous materials that have a high potential feedstock for biogas production. In the past, utilization of OPDC was limited to use in oil palm plantations for mulching and soil conditioning. Recently, many researchers have studied the suitability of OPDC as co-boiler fuel [6, 7], animal feed [8], fish feed [9], composting material and enzymes [10, 11], cellulase and polyoses [12], and as a source of biofuel (biogas, hydrogen, and methane) production [7, 13]. However, its economic value is low and it is, therefore, not considered to possess an attractive potential for use as substrate for bioactive compounds production. As the major components of OPDC are lignin (30.66%), cellulose (21.61%), and hemicellulose (3.94%) [12], it can be utilized as an alternate low-cost substrate for lignocellulosic enzyme production that acts corporately to degrade the components of lignocellulose. Moreover, OPDC contains organic matters and nutrients (nitrogen, carbon sulfur, phosphate, potassium, calcium, magnesium, boron, manganese, copper iron, and zinc) [12] that are essential for the growth of *Actinobacteria* during ligninolytic enzyme and bioactive compounds production [14].

Actinomycetes play a pivotal role in maintaining a satisfactory biological balance in soil, largely because of their ability to produce antibiotics and other secondary metabolites [15, 16]. Among actinomycetes, *Streptomyces* species are well known for their essential role in the naturally occurring degradation of lignocellulose, which is enabled by the secretion of extracellular of lignocellulosic (cellulase and xylanase) [17, 18] and ligninolytic (lignin peroxidase, manganese peroxidase, and laccase) [14, 19] enzymes. Moreover, *Streptomyces* are a major source for production of antibiotics [20] and bioactive compounds [21] with their potential applications in agriculture, medicine, and the food industry. Numerous studies on antimicrobial production by *Streptomyces* were reported using synthetic media [22–26]. In particular, the glucose yeast-malt extract (GYM) medium has been shown to be a good medium for growth and antifungal production of *S. philanthi* RM-1-138 [27–30], but it has never been reported using OPDC as the medium. Therefore, this work contributes to knowledge on an alternative potential of OPDC as a low-cost substrate for bioactive compound production by *S. philanthi* RM-1-138 using solid-state fermentation (SSF). SSF using agro-industrial wastes is an attractive and cost-effective option because it represents higher productivity with simpler operation when compared with submerged fermentation. This research aimed to investigate the effectiveness of OPDC as a substrate in the SSF system for bioactive compounds production by *S. philanthi* RM-1-138 and evaluation of its efficacy against both plant pathogenic fungi and bacteria.

2 Materials and methods

2.1 Microorganisms, media, and cultivation conditions

Streptomyces philanthi RM-1-138 was previously isolated and characterized as described by Boukaew et al. [27] and grown on GYM agar at room temperature (28 ± 2 °C) for 10 days before use.

Curvularia oryzae and *Ganoderma boninense* were oil palm pathogenic fungi that obtained from the Suratthani Oil Palm Research Center, Department of Agriculture, Ministry of Agriculture and Cooperative, Thailand and Biohythane Laboratory at Prince of Songkla University (PSU), Thailand, respectively. *Ceratocystis paradoxa* TT1 was isolated from the natural fermented oil palm trunk [31] and reported to cause black seed rot disease in oil palm sprouted seeds [32]. They are known to cause significant losses in oil palm crops in Southeast Asia, particularly in Thailand, Malaysia, and Indonesia [33, 34]. The fungal strains were grown on potato dextrose agar (PDA) slants at room temperature (28 ± 2 °C) for 3–5 days and kept at 4 °C. They were sub-cultured freshly for use in each experiment.

Plant pathogenic bacteria *Xanthomonas axonopodis* pv. *glycines*, *Xanthomonas oryzae* pv. *oryzae*, and *Xanthomonas campestris* pv. *campestris* were obtained from the Department of Plant Pathology, Faculty of Agriculture, at Kasetsart University (KU), Thailand. They are known to cause significant losses in many crop plants worldwide. The bacterial strains were grown on nutrient agar (NA) slants at room temperature (28 ± 2 °C) for 24–48 h and kept at 4 °C. The inoculum was prepared by culturing the bacterium in nutrient broth (NB) for 24 h on a rotary shaker (at 150 rpm). The concentration of the inoculum was then adjusted to 10^6 CFU mL⁻¹ before used.

2.2 Oil palm decanter cake and its characteristics

Oil palm decanter cake (OPDC) and palm pressed fiber (PPF) were collected from a palm oil mill (Laff Tavee Palm Co., Ltd.) in Satun province, Southern Thailand. OPDC was dried in an oven at 95 °C until the moisture content was lower than 5% [35]. Characteristics of OPDC were determined for chemical oxygen demand (COD), total solid (TS), total suspended solids (TSS), total phosphorous (TP), and phosphate following the Standard Method [36]. Total Kjeldahl nitrogen (TKN) was analyzed using Kjeldahl method [37]. Temperature and pH were measured using thermometer and pH meter, respectively. Glucose, xylose, fructose, and arabinose were determined using high-performance liquid chromatography (HPLC) [38].

2.3 Enzyme assays

2.3.1 Raw materials for enzyme production of *S. philanthi* RM-1-138

Solid-state fermentation of *S. philanthi* RM-1-138 grown in oil palm decanter cake (OPDC), palm pressed fiber (PPF), and OPDC + PPF (1:1) as substrates for the production of cellulase, xylanase, chitinase, and β -1,3-glucanase was investigated.

2.3.2 Cellulase and xylanase activity

The cellulase and xylanase activity were determined according to Bailey et al. [39]. Quantitation of reducing sugar released from xylan or carboxymethylcellulose (CMC) prepared in 50 mM acetate buffer pH 5.0 with 0.1 mL of the appropriately diluted enzyme to give an absorbance reading below 0.7. The enzyme-substrate mixture was incubated at 50 °C for 5 min. The released reducing sugars were determined by the use of 3,5-dinitrosalicylic acid (DNS) method with xylose or glucose used as standards for xylanase and cellulase activities, respectively [40]. One unit of xylanase or cellulase is defined as the amount of enzyme that liberates 1 μ mol of xylose or glucose equivalents per minute, respectively.

2.3.3 Chitinase and β -1,3-glucanase activity

Chitinase and β -1,3-glucanase activity were measured using colloidal chitin and laminarin as the substrates, respectively following the DNS method [40]. The reaction mixture of chitinase and β -1,3-glucanase activity was prepared according to the method of Chairin and Petcharat [41] with some modification. Briefly, the reaction mixture contained 250 μ L of crude sample and 1% (w/v) substrate in 250 μ L of 50 mM potassium phosphate buffer (KPB) pH of 7.0 for colloidal chitin and 50 mM acetate buffer pH of 6.0 for laminarin and incubated for 30 min in 50 °C water bath. Reducing sugar released in the mixtures was determined by recording the absorbance at 570 nm for chitinase and 550 nm for β -1,3-glucanase. One unit (U) of chitinase activity was defined as releasing 1 μ mol of N-acetyl-D-glucosamine from the substrate per minute and one unit of β -1,3-glucanase activity was defined as releasing 1 μ mol of glucose from the laminarin per minute.

2.4 Optimization of raw materials and environmental condition for enzymes production of *S. philanthi* RM-1-138

2.4.1 Effect of raw materials

The effect of raw materials, OPDC, PPF, and OPDC mixed with PPF (1:1), on enzyme production of *S. philanthi*

RM-1-138 was investigated. Five milliliter aliquots seed culture (10^7 spore mL⁻¹) of the strain RM-1-138 was transferred into 50 g each of the sterilized (121 °C/15 min) raw materials (with moisture content of 60%; initial pH adjusted to 7.0) and incubated at room temperature (28 ± 2 °C). After 12 days incubation, the enzymes were extracted from the cultivated materials by adding 100 mL of 0.1 M phosphate buffer (pH 7.0) and placed on a rotary shaker (at 150 rpm) for 2 h [42, 43]. The suspension was centrifuged (at $8880 \times g$ for 20 min), then filtered through a 0.45 μ m Millipore membrane to obtain the supernatant. The samples were taken every 2 days to measure the enzyme activities. The supernatant was determined for enzyme activity. The raw materials that exhibited the highest enzyme activity were selected for further studies.

2.4.2 Effect of moisture content

The effect of moisture contents (30, 40, 50, 60, and 70%) of the selected raw material (results from previous experiment) on enzyme production from *S. philanthi* RM-1-138 was studied. Five milliliter aliquots seed culture of the strain RM-1-138 was transferred into 50 g of each sterilized selected raw materials with pH adjusted to 7.0 and incubated at room temperature (28 ± 2 °C) for 10 days. The moisture content that exhibited the highest enzyme activity was selected for further studies.

2.4.3 Effect of initial pH

The effect of initial pH (6.0, 7.0, and 8.0) on production of enzymes by *S. philanthi* RM-1-138 was investigated. Five milliliter aliquots seed culture of the strain RM-1-138 was transferred into 50 g of the sterilized optimum raw materials (result from the previous experiment) and incubated at room temperature (28 ± 2 °C) for 10 days. The initial pH that exhibited the highest enzyme activity was selected for further studies.

2.5 Testing for the presence of bioactive compounds in the culture filtrate of *S. philanthi* RM-1-138 against oil palm pathogenic fungi and bacteria

The culture filtrate of *S. philanthi* RM-1-138 (culture filtrate RM-1-138) grown in the optimum raw materials and condition (results from the optimization studies) was tested for its efficacy against both oil palm pathogenic fungi (*C. oryzae*, *G. boninense*, and *C. paradoxa* TT1) and bacteria (*X. axonopodis* pv *glycines*, *X. oryzae* pv *oryzae*, and *X. campestris* pv *campestris*). The experiment was carried out as above and the culture filtrate was extracted from the cultivated medium (as described above) at the optimum

incubation time and used for the antimicrobial assay. The positive results on antifungal or antibacterial assay would indicate the presence of bioactive compounds in the culture filtrate RM-1-138.

2.5.1 Antifungal assay

The culture filtrate RM-1-138 (1 mL) was mixed with 9 mL melted sterile PDA (at 60 °C) and poured onto a 9 cm diameter culture plate, while distilled sterilized water (1 mL) mixed with 9 mL melted sterile PDA at an equivalent amount was used as a control. A 5 mm diameter plug of mycelia was cut from a 3-day old in each oil palm pathogens colony and transferred onto the center of the test agar plates. The cultures were further incubated at room temperature (28 ± 2 °C) for 2 days of *C. paradoxa* TT1, 4 days of *C. oryzae*, and 7 days of *G. boninense*. The experiment was conducted in three replications. The colony size of each treatment was recorded and the percentage inhibition of hyphal growth was calculated using the equation: percentage of inhibition = $[(\text{control-treatment}) / \text{control}] \times 100$ [29].

2.5.2 Antibacterial assay

Agar well diffusion assay was used for the detection of antibacterial activity. NA plates containing 10^6 CFU per mL of bacteria pathogen were prepared. A well with a diameter of 6 mm was then cut into the agar using a sterile cork-borer. A droplet of agar was added to the well in order to seal it to avoid leakage. Then, 100 μ L of the culture filtrate RM-1-138 was added to the well and allowed to diffuse into the agar during a 5-h pre-incubation period at room temperature, followed by incubation at room temperature (28 ± 2 °C) for 48 h. The antibacterial zone was recorded.

2.6 Statistical analysis

The data were submitted to analyses of variance using Statistical Package for the Social Sciences (SPSS) ver. 26 (IBM Corp; IBM SPSS Statistics for Windows, ver. 26.0, Armonk, NY). A *P* value < 0.05 was considered significant.

3 Results

3.1 Characteristics of oil palm decanter cake (OPDC)

Raw OPDC had an acidic pH (5.33) and black color. The characteristics of OPDC (Table 1) indicated that the high values of organic matter (9828 mg kg⁻¹, COD) showed the possibility to be used as a carbon source for the growth of microorganisms. Total solids (TS) and suspended solids (TSS) (21,762 and 18,330 mg kg⁻¹, respectively). It also

contained nitrogen (TKN of 1443 mg kg⁻¹ and NH₄-N of 358.8 mg kg⁻¹) as well as various sugars: glucose, xylose, fructose, and arabinose (1396, 1154, 319, and 324 mg kg⁻¹, respectively). These chemical composition showed the possibility that OPDC could be used as substrate for the growth of microorganisms.

3.2 Optimization of raw materials and environmental condition for enzymes production of *S. philanathi* RM-1-138

3.2.1 Effect of raw materials

Three sets of raw materials, OPDC, PPF, and OPDC + PPF (1:1), were used to study their effects on xylanase, cellulase, chitinase, and β -1,3-glucanase production of *S. philanathi* RM-1-138. They showed significant differences in enzyme production (Fig. 1) with the highest values from OPDC followed by PPF and OPDC + PPF (1:1) after 12 days incubation. OPDC as the substrate gave the higher enzyme activity of xylanase (Fig. 1A), cellulase (Fig. 1B), and chitinase (Fig. 1C) (5.21 ± 0.26 , 3.75 ± 0.38 , and 0.48 ± 0.01 U mL⁻¹, respectively) at 10 days incubation. In addition, the production of β -1,3-glucanase was not detected in all raw materials. Therefore, the OPDC was the selected raw material for further studies.

3.2.2 Effect of moisture content and initial pH

The enzyme assay was employed to study the effect of moisture contents (30–70%) and initial pH (6–8) in OPDC culture of *S. philanathi* RM-1-138 on the production of xylanase,

Table 1 Characteristics and chemical composition of oil palm decanter cake (OPDC) for bioactive compounds production from *Streptomyces philanathi* RM-1-138

Component	Unit	Decanter cake
Color		Black
pH		5.33
Total Kjeldahl nitrogen (TKN)	mg kg ⁻¹	1,443 \pm 6.3
Ammonium-nitrogen (NH ₄ -N)	mg kg ⁻¹	358.8 \pm 2.8
Total phosphorous	mg kg ⁻¹	553.8 \pm 2.8
Phosphate	mg kg ⁻¹	276.9 \pm 7.1
Chemical oxygen demand (COD)	mg kg ⁻¹	9,828 \pm 70.7
Total solids (TS)	mg kg ⁻¹	21,762 \pm 14.1
Total suspended solids (TSS)	mg kg ⁻¹	18,330 \pm 4.2
Glucose	mg kg ⁻¹	1,396 \pm 0.04
Xylose	mg kg ⁻¹	1,154 \pm 0.03
Fructose	mg kg ⁻¹	319 \pm 0.02
Arabinose	mg kg ⁻¹	324 \pm 0.04

Data are the mean of three replicates \pm standard deviation (SD). Units are in mg kg⁻¹ except color and pH

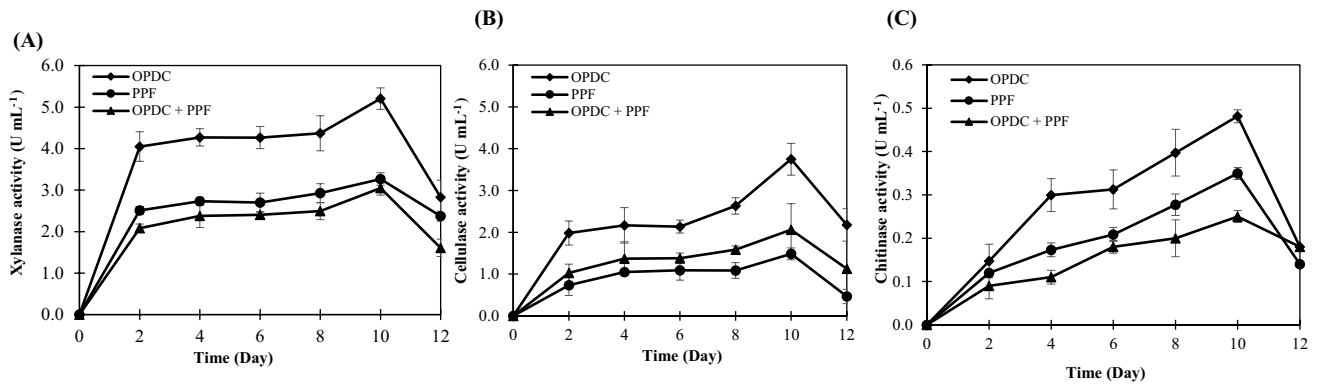


Fig. 1 Time course on the production of xylanase (A), cellulase (B), and chitinase (C) from *Streptomyces philanthi* RM-1-138 in oil palm decanter cake (OPDC) (square), palm pressed fiber (PPF) (circle),

and OPDC + PPF (triangle) and adjusted moisture content to 60% after cultivation for 12 days using solid-state fermentation. Data are the mean of three replicates \pm standard deviation (SD)

cellulase, chitinase, and β -1,3-glucanase after incubating for 10 days (Fig. 2). The increasing amounts of moisture content from 30 to 60% resulted in a significant increase in the production of xylanase and cellulase by 2.34 and 2.45 fold (from 2.53 ± 0.12 to 5.91 ± 0.26 U mL⁻¹ and 1.61 ± 0.38 to 3.95 ± 0.38 U mL⁻¹, respectively). The optimal moisture content for enzyme production was found to be 60%. Substrate moistened at this level afforded high xylanase, cellulase, and chitinase activity values of 5.91 ± 0.26 , 3.95 ± 0.38 , and 0.56 ± 0.01 U mL⁻¹, respectively (Fig. 2A), indicating this moisture content being favorable for *S. philanthi* RM-1-138 growth in OPDC medium. However, β -1,3-glucanase activity was not detected at all in every initial moisture content tested.

The impact of the initial pH (6–8) of OPDC on the production of xylanase, cellulase, chitinase, and β -1,3-glucanase from *S. philanthi* RM-1-138 was studied in the optimum OPDC culture (Fig. 2B). The highest xylanase, cellulase, and chitinase activities were obtained at an initial pH of 7.0 (6.03 ± 0.25 , 4.79 ± 0.76 , and 0.76 ± 0.02 U mL⁻¹, respectively) but no significant differences ($P > 0.05$) of xylanase (5.87 ± 0.10 U mL⁻¹) and chitinase (0.61 ± 0.02 U mL⁻¹) activities at the initial pH of 8.0.

3.3 Testing for the presence of bioactive compounds in the culture filtrate of *S. philanthi* RM-1-138 against oil palm pathogenic fungi and bacteria

The presence of bioactive compounds in the culture filtrate of *S. philanthi* RM-1-138 produced in the optimal OPDC medium on the growth of the three oil palm pathogenic fungi (*C. oryzae*, *C. paradoxa* TT1, and *G. boninense*) and bacteria (*X. axonopodis* pv. *glycines*, *X. oryzae* pv. *oryzae*, and *X. campestris* pv. *campestris*) was investigated (Table 2). The mycelial growth of the three oil palm pathogenic fungi

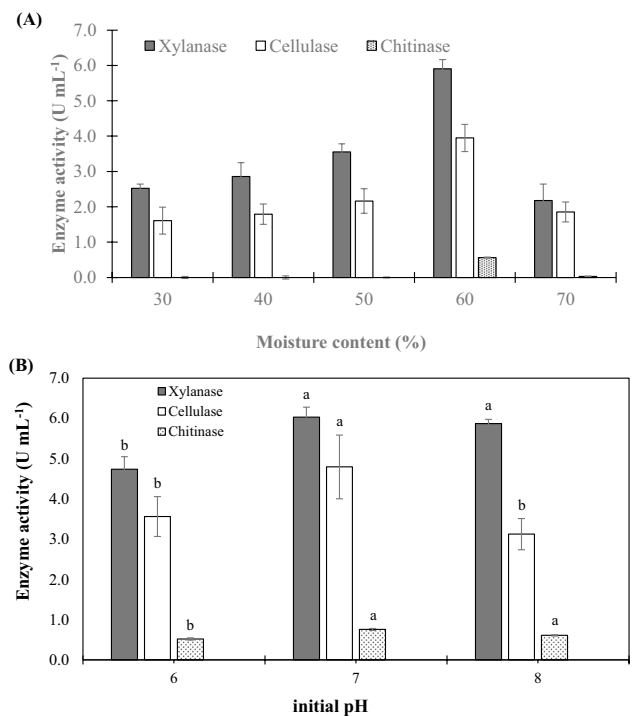


Fig. 2 The effect of initial moisture content (A) and initial pH (B) in oil palm decanter cake (OPDC) medium on the production of xylanase, cellulase, and chitinase from *Streptomyces philanthi* RM-1-138 after cultivation for 10 days. Data are the mean of three replicates \pm standard deviation (SD). Values with the same letter are not significantly different (ANOVA, $P > 0.05$; Duncan's multiple range test)

was inhibited with the most pronounced inhibitory effect (74.33%) was against *G. boninense* followed by against *C. oryzae* (64.67%) and *C. paradoxa* TT1 (58.33%) (Table 2A). For agar well diffusion assay on pathogenic bacteria tested, no significant differences ($P > 0.05$) on pathogenic bacteria were observed. The bioactive compounds were shown to inhibit the growth of each plant pathogenic bacteria in the

inhibition zone range of 18.33 to 19.17 mm (Table 2B). The most pronounced inhibitory effect was against *X. axonopodis* pv. *glycines* (19.17 mm) followed by *X. oryzae* pv. *oryzae* (18.50 mm), and *X. campestris* pv. *campestris* (18.33 mm).

4 Discussion

The antimicrobial metabolites produced by *Streptomyces* species are known to be efficient against plant pathogenic fungi [44, 45] and bacteria [46–48]. To substitute the mostly used expensive synthetic medium, oil palm decanter cake (OPDC) was proposed to be used as substrate for production of enzymes under solid-state fermentation. In this study, the efficacy of the antimicrobial metabolites produced in OPDC of *S. philanthi* RM-1-138 was evaluated against three strains of oil palm pathogenic fungi (*Curvularia oryzae*, *Ganoderma boninense*, and *Ceratocystis paradoxa* TT1) and bacteria (*Xanthomonas axonopodis* pv. *glycines*, *X. oryzae* pv. *oryzae*, and *X. campestris* pv. *campestris*).

Initial moisture contents and pH of the substrate are known to critically influence microorganism growth and bioactive compounds production in solid-state fermentation (SSF) [49, 50]. The highest enzymes activity was noted in OPDC followed by PPF after 12 days incubation. OPDC was high in organic matters (9828 mg kg⁻¹ COD, 21,762 mg kg⁻¹ TS and 18,330 mg kg⁻¹ TSS). The concentration of nitrogen (1443 mg kg⁻¹ TKN), glucose and xylose (1396 and 1154 mg kg⁻¹, respectively) were similar while the amount of fructose and arabinose were much lower (319, and 324 mg kg⁻¹, respectively). The characteristics of OPDC obtained are expected to deviate highly from the literature as it varies with the quality of palm fruits, processing techniques, quality control of individual mills, crop seasons, and

other factors. Besides, TKN, COD, TS, and TSS of OPDC are observed to be relatively lower than those reported elsewhere [12, 51–53] as it depends on the sampling point of the substrate where the settling of solids occurred. With its high TS content, OPDC is a promising material for use as a renewable energy source through the SSF process for production of lignocellulosic enzymes and bioactive compounds. Although the weight substrates (50 g) content of the two raw materials was similar, OPDC is more easily digested than PPF. This probably explains the better bioactive compounds production in OPDC. The results indicated that the differences in compositions of raw materials had influenced bioactive compounds production by *S. philanthi* RM-1-138.

OPDC used as a sole substrate afforded the highest levels of xylanase (5.21 ± 0.26 U mL⁻¹), cellulase (3.75 ± 0.38 U mL⁻¹), and chitinase (0.48 ± 0.01 U mL⁻¹) production than the PPF and the mixed substrates. It is worth of noting that the strain RM-1-138 showed multiple (endo- and exo-cellulase as well as xylanase) enzymatic activities that lends them interesting as sources of biocatalysts for lignocellulose conversion. Very few works investigated so far cellulase, xylanase, and chitinase production in *S. philanthi* RM-1-138, even if cellulolytic activity has been reported in different *Actinobacteria*, such as *Streptomyces*, *Thermoactinomyces*, and *Cellulomonas*. The highest xylanase activity (5.21 ± 0.26 U mL⁻¹) achieved by *S. philanthi* RM-1-138 in the present work was higher than the activity from *S. flavogriseus* AE63X (0.95 U mL⁻¹) [54], *Streptomyces* strain C1-3 (0.6 U mL⁻¹) [55], and *Streptomyces* strain AMT-3 (0.17 ± 0.02 U mL⁻¹) [56]. In addition, the cellulase activity (3.75 ± 0.38 U mL⁻¹) was much higher than the cellulase activity (0.87 ± 0.19 U mL⁻¹) from *S. flavogriseus* AE64X [54]. However, the chitinase activity (0.48 ± 0.01 U mL⁻¹) using OPDC medium was lower than previously reported with cultivated in a basal medium containing 0.05% (w/v) MgSO₄, 0.02% (w/v) K₂HPO₄, 0.03% (w/v) KH₂PO₄, and 0.001% (w/v) each of FeSO₄, ZnSO₄, and MnCl₂ (0.53 U mL⁻¹) [57], than that of *S. thermocarboxydus* TKU045 (52.985 U mL⁻¹) [58], and that of *S. vinaceusdrappus* S5MW2 (14.5 ± 0.4 U mL⁻¹) [59]. It is worth of noting that the values of these enzyme activities may be obtained from the differences in the analytical methods such as the substrate and incubation condition.

The presence of water in the substrate makes the nutrients more easily accessible for bacterial growth. Moreover, water has an impact on physico-chemical properties of the substrate, which in turn affect enzyme production [60]. Too much water adversely affects oxygen diffusion in the substrate [49]. In this work, the initial moisture contents of the substrate were adjusted to 30–70% in separate experiments before inoculation with the strain RM-1-138. Clearly, the optimum initial moisture level was 60%. Substrate moistened at this level afforded a high cellulase, xylanase, and

Table 2 Antimicrobial effect of culture filtrate of *Streptomyces philanthi* RM-1-138 grown on optimal oil palm decanter cake (OPDC) against three strains each of oil palm pathogenic fungi (A) and bacteria (B)

m	
Fungal pathogen	Inhibition percentage ± SD
<i>C. oryzae</i>	64.67 ± 3.79 ^b
<i>C. paradoxa</i> TT1	58.33 ± 5.51 ^b
<i>G. boninense</i>	74.33 ± 4.93 ^a
(B)	
Bacterial pathogen	Inhibition zone (mm) ± SD
<i>X. axonopodis</i> pv. <i>glycines</i>	19.17 ± 2.40 ^a
<i>X. oryzae</i> pv. <i>oryzae</i>	18.50 ± 1.48 ^a
<i>X. campestris</i> pv. <i>campestris</i>	18.33 ± 0.60 ^a

Data are the mean of three replicates ± standard deviation (SD). Values with the same letter are not significantly different (ANOVA, $P > 0.05$; Duncan's multiple range test)

chitinase activity (5.91 ± 0.26 , 3.95 ± 0.38 , and 0.56 ± 0.01 U mL⁻¹, respectively) at 10 days. This result was lower than that for xylanase and cellulase production by *A. niger* (65%) [61], (77.67%) [62], (75%) [63], *Penicillium canescens* (83%) [64], *Melanocarpus albomyces* IIS-68 (67%) [65], and *Thermoascus aurantiacus* (81%) [66], but higher than by *Chaetomium globosum* (50%) [67]. This may be due to the different culture media and microorganisms. Moisture levels above 60% reduced enzyme production as the substrate became water logged [68]. High moisture content is known to reduce voidage or porosity of substrates, causes particles to stick together, and adversely impacts oxygen transfer to the microorganisms [46, 69]. This explains the effects of elevated levels of moisture.

As a general practice, the pH in SSF is almost never controlled during fermentation, and only the initial pH of the substrate is adjusted before inoculation. Most substrates such as wheat bran, rice husk, rice bran, spent brewing grain, coconut oil cake, palm kernel cake, sesame oil cake, jackfruit seed powder, and olive oil cake used in SSF are known to possess an excellent buffering capacity [46, 69]. The results revealed that the optimum pH of the medium for enzyme production was 7.0. It could be speculated that SSF contributed to a better buffering capacity. Medium pH is very important in nutrient absorption and growth of bacteria, stimulation of enzyme production via signaling pathways, and release of extracellular enzymes [70].

In the in vitro assay, *S. philanthi* RM-1-138 was able to produce both antifungal and antibacterial metabolites compounds using OPDC as substrate medium, which greatly inhibited the plant pathogenic growth of three strains each of fungi (*C. oryzae*, *C. paradoxa* TT1, and *G. boninense*) and bacteria (*X. axonopodis* pv. *glycines*, *X. oryzae* pv. *oryzae*, and *X. campestris* pv. *campestris*) tested. In this study, we demonstrated that the antifungal compounds of *S. philanthi* RM-1-138 can significantly ($P < 0.05$) affect against the three strains of oil palm pathogen. Among the three strains of oil palm fungal pathogen tested, antifungal compounds exhibit the strongest inhibition against *G. boninense* (74.33%). It is much higher than the antifungal compounds produced in the synthetic medium (56.64% inhibition) reported by Shariffah-Muzaimah et al. [71] but lower than those against *G. boninense* (92.04% inhibition) and *C. oryzae* (84.60% inhibition) reported by Pithakkit et al. [33]. Among the three bacterial strains tested, the antibacterial compounds was not significantly difference ($P > 0.05$) against *X. axonopodis* pv. *glycines* (19.17 mm), *X. oryzae* pv. *oryzae* (18.50 mm), and *X. campestris* pv. *campestris* (18.33 mm). It can be seen that the antibacterial compounds obtained in OPDC medium is much higher than the efficacy reported by Okudoh and Wallis [72], Jaivel et al. [73], Kováčsová et al. [74], and He et al. [75] (15–16 mm) but lower than those against *X. oryzae* pv. *oryzae* (25

mm) reported by Hastuti et al. [76]. The results from antifungal and antibacterial actives indicated that OPDC has high potential for utilization as an alternative low-cost SSF medium for the production of both antimicrobial compounds by *S. philanthi* RM-1-138. This strain was reported to have several mechanisms such as bioactive and volatile compounds by which it inhibits plant pathogens as reported for many *Streptomyces* species, such as, *S. globisporus* JK-1 [22], *S. philanti* RM-1-138 [27–30], and *S. mycarofaciens* SS-2-243 [30]. However, to our knowledge, this study is the first one revealing the utilization of OPDC, a waste product of palm oil processing, as an alternative low-cost SSF medium for the production of the bioactive compound by *S. philanthi* RM-1-138. Based on the in vitro experiment, the *S. philanthi* RM-1-138 could grow in OPDC and secreted both antifungal and antibacterial compounds into the medium.

In conclusion, the results obtained in this study clearly indicated that OPDC had high potential for utilization as an alternative low-cost SSF medium for the production of both antifungal and antibacterial compounds by *S. philanthi* RM-1-138. The antimicrobial compounds have high efficacy against three strains of oil palm pathogenic fungi and bacteria.

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Declarations

Human and animals rights No human and/or animal participants were involved in this research.

Competing interests The authors declare no competing interests.

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