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RESEARCH

Efficacy of the antifungal metabolites of *Streptomyces philanthi* RL-1-178 on aflatoxin degradation with its application to prevent aflatoxigenic fungi in stored maize grains and identification of the bioactive compound

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Abstract
Aflatoxin B_1 is a potent carcinogen produced by *Aspergillus flavus (A. flavus)* and *Aspergillus. parasiticus (A. parasiticus)*, mainly during grain storage. The efficacy of the freeze-dried culture filtrate of *Streptomyces philanthi (S. philanthi)* strain RL-1-178 (DCF) on degradation of aflatoxin B_1 (AFB_1) were evaluated and its bioactive compounds were identified. The DCF at a concentration of 9.0% (w/v) completely inhibited growth and AFB_1 production of *A. parasiticus* TISTR 3276 and *A. flavus* PSRDC-4 after 7 days tested in yeast-extract sucrose (YES) medium and on stored maize grains after 28 and 14 days incubation, respectively. This indicated the more tolerance of *A. parasiticus* over *A. flavus*. The DCF and bacterial cells of *S. philanthi* were capable to degrade AFB_1 by 85.0% and 100% for 72 h and 8 days, respectively. This confirmed the higher efficacy of the DCF over the cells. After separation of the DCF on thin-layer chromatography (TLC) plate by bioautography bioassay, each active band was identified by liquid chromatography—quadrupole time of flight mass spectrometer (LC-Q-TOF MS/MS). The results revealed two compounds which were identified as azithromycin and an unknown based on mass ions of both ESI^+ and ESI^- modes. The antifungal metabolites in the culture filtrate of *S. philanthi* were proved to degrade aflatoxin B_1. It could be concluded that the DCF may be applied to prevent the growth of the two aflatoxin-producing fungi as well as the occurrence of aflatoxin in the stored maize grains.

Keywords Aflatoxin B_1 · *Streptomyces philanthi* · Maize grains · AFB_1 degradation · Antifungal compounds

Introduction

The colonization of maize grains by *Aspergillus flavus (A. flavus)* and *Aspergillus. parasiticus (A. parasiticus)* (Link ex Fr. and Speare, respectively; teleomorphs: *Petromyces flavus* and *P. parasiticus*) (Fountain et al. 2014, 2015; Okun et al. 2015; García-Díaz et al. 2020) results in contamination of their derived feed, and food products with aflatoxins (AFs) (Diener et al. 1987). Aflatoxin B_1 (AFB_1) is known as the most toxic aflatoxin, and several studies have demonstrated its hepatotoxic, carcinogenic, and mutagenic effects on human and animals (Guengerich et al. 1996; Hussein and Brasel 2001; Farzaneh et al. 2012; Afsharmanesh et al. 2018).

During the last decades, several studies have been dedicated to physical and chemical strategies for the reduction of AFs in crops, foods and feeds (Kabak et al. 2006; Afsharmanesh et al. 2018; Sipos et al. 2021; Nji et al. 2022). Nevertheless, none of these strategies completely fulfils the necessary efficacy, safety and cost requirements (Mishra and Das 2003; Zhao et al. 2011). These disadvantages encouraged a recent emphasis on the biological degradation of aflatoxins. Biodegradation of aflatoxins using microorganisms,

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is one of the environmentally friendly strategies to reduce or eliminate the possible contaminations of AFs in foods and feeds (Farzaneh et al. 2012; Sangare et al. 2014; Afsharmanesh et al. 2018). There are two key directions in control of aflatoxin contamination: preventing the growth of toxigenic aflatoxin-producing fungi, namely prevention and if contamination occurs, then detoxify aflatoxin-contaminated commodities by removing the toxic compounds (Xia et al. 2017; Shu et al. 2018). Over the past decades, some bacterial species have been known to degrade aflatoxin, which include *Nocardia corynebacterioides* (Ciegler et al. 1966; Hormisch et al. 2004), Rhodococcus erythropolis *(R. erythropolis)*, *Mycobacterium fluorantheniorans* (Teniola et al. 2005), Bacillus licheniformis *(B. licheniformis)* (Petchkongkaew et al. 2008; Rao et al. 2017), Bacillus. Subtilis *(B. subtilis)* (Petchkongkaew et al. 2008; Gao et al. 2011; Farzaneh et al. 2012; Siahmoshteh et al. 2017; Xia et al. 2017; Afsharmanesh et al. 2018; Wang et al. 2019; Suresh et al. 2020), Bacillus velezensis (*B. velezensis)* (Shu et al. 2018; Wang et al. 2021), *Bacillus. amyloliquefaciens* (Siahmoshteh et al. 2017), Bacillus. Megaterium *(B. megaterium)* (Wang et al. 2021), *Pseudomonas aeruginosa* (Sangare et al. 2014). In addition, some *Streptomyces* sp. strains also appeared as valuable candidates for controlling filamentous fungal growth and inhibiting mycotoxin production (Harkai et al. 2016; Campos-Avelar et al. 2021). The development of efficient antifungal microbial agents could be an alternative method to control the fungi.

Actinobacteria present an interesting, natural, and cost-effective alternative for the effective biodegradation of AFs (Oliveira et al. 2013). Many species of actinomycetes, particularly those belonging to the genus *Streptomyces* are largely researched for their ability to produce numerous molecules of interest, namely, antibiotics (Igarashi et al. 2005; Quinn et al. 2020), antifungal compounds (Boukaew et al. 2017, 2020a, 2020b, 2021; Chen et al. 2016; Li et al. 2011; Shakeel et al. 2016), and hydrolytic enzymes (glucanase, chitinase) (Prapagdee et al. 2008; Boukaew et al. 2016; Vaz-Jauri et al. 2016), which provide them with strong antagonistic capacities against fungal development. The impact of *Streptomyces* sp. on toxigenic aflatoxin-producing fungi growth has already been assessed, in addition to their ability to degrade AFB_1 (Verheecke et al. 2014; Harkai et al. 2016; Campos-Avelar et al. 2021) and to inhibit its production. Indeed, some *Streptomyces* strains produce aflastatin A, blasticidin A, and dioctatin A, three molecules that inhibit the AFs biosynthetic pathway (Sakuda et al. 1996; Sakuda 2010). In addition, a *Streptomyces. roseolus* strain was found to reduce AFB_1 production by inhibiting aflatoxin gene cluster expression in *A. flavus* (Caceres et al. 2018). *Streptomyces* isolates IX45 can effectively restrict the growth of *A. flavus* growth and remove AFB_1 production with 31% as previously described by Campos-Avelar et al. (2021), while 88.34% AFB_1 degradation by cell-free supernatant of *S. cacaoi* sub sp. *asoensis* K234 was also observed by Harkai et al. (2016). In our previous studies, the antifungal compounds produced in tuna condensate waste medium of the strain Streptomyces philanthi *(S. philanthi)* showed high efficacy to inhibit mycelial growth and AFB_1 production of *A. flavus* and *A. parasiticus* (Boukaew et al. 2020b, c), however, the aflatoxin B_1 degradation was not investigated. As a consequence, the efficacy of antifungal metabolites of *S. philanthi* RL-1-178 on AFB_1 degradation will be investigated in this study.

The objectives of the present study were to (i) investigate the efficacy of antifungal compounds produced by *S. philanthi* on growth and aflatoxin B_1 production of the two aflatoxin-producing fungi, (ii) to evaluate the efficacy of antifungal compounds of *S. philanthi* as potential biocontrol agents of maize grain pathogenic fungi, (iii) to evaluate degradation efficiency of antifungal compounds and *S. philanthi* on AFB_1 and (iv) to identify the active compound responsible for antifungal activity.

Materials and methods

Microorganisms and preparation of freeze-dried culture filtrate

The antagonistic strain *S. philanthi* RL-1-178 was previously isolated from the rhizosphere of chili pepper in southern Thailand (Boukaew et al. 2011). The culture filtrate of *S. philanthi* RL-1-178 was prepared by inoculating 10% (v/v) aliquots of the seed culture into 5 l bioreactor (New Brunswick™ BioFlo® 415 Sterilize-in-Place (SIP) Fermentor, Eppendorf North America) containing 4 l tuna condensate medium (pH was adjusted to 7.0 before autoclaving) and stirred (150 rpm) at 30 °C for 10 days (Boukaew et al. 2020c). Then, the culture broth was centrifuged (8880 × *g* for 20 min) and filtered through a 0.45 mm Millipore membrane. After that, the culture filtrate was freeze-dried by vacuum freeze-dryer at the Office of Scientific Instrument and Testing, Prince of Songkla University (PSU) to obtain the freeze-dried culture filtrate from *S. philanthi* RL-1-178 (called DCF).

Aflatoxigenic fungal strains and spore inoculum preparation

The *A. parasiticus* strain TISTR 3276 and *A. flavus* strain PSRDC-4 were previously found to be as high aflatoxin producers (Boukaew et al. 2020b,c). They were cultivated on potato dextrose agar (39 g l^{-1}; Difco Laboratory) at 30 °C. Spores were collected in 5 ml water from10-day-old culture and counted using a hematocytometer. The inoculum was

prepared by dilution in sterilized distilled water to achieve the required concentration.

Efficacy of the DCF concentration against the two aflatoxigenic fungal strains

The efficacy of the DCF concentration against *A. parasiticus* TISTR 3276 and *A. flavus* PSRDC-4 was investigated. One gram of the DCF was dissolved in 10 ml of dimethyl sulfoxide (DMSO) and filtered through a 0.45 mm Millipore membrane. The DCF solution for each treatment (at 0.2, 0.4, 1.0, 2.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0% (w/v)) were incorporated into melted sterile yeast-extract sucrose (YES) medium at a final concentration of 45 ml. DMSO added to YES medium at equivalent amounts was used as a control. Fifty µl of spore inoculum (10^5 spore mL^{-1}) of each aflatoxigenic fungal strain was transferred into each flask and incubated on a rotary shaker (150 rpm) at 30 °C for 7 days (Komala et al. 2012). After 7 days cultivation, the mycelial mats were washed (with sterile water) and dried at 80 °C until constant weight (Tolouee et al. 2010; Sangmanee and Hongpattarakere 2014). The inhibition of hyphal growth was calculated as: Percentage of inhibition = {[(Control-treatment)/Control] × 100}.

For aflatoxin B_1 production, samples after treatment were extracted with chloroform according to the Association of Official Analytical Chemists (Tosch et al. 1984) and AFB_1 concentration was determined as previously described by Boukaew et al. (2020c). For each treatment, three replicates were conducted. AFB_1 concentration was determined by indirect competitive ELISA (Enzyme-linked Immunosorbent assay) using a ScreenEZ® Aflatoxin ELISA test kit (Siam Inter Quality Co., Ltd., Thailand).

Evaluation of the DCF efficiency on growth and aflatoxin B_1 production of the two aflatoxigenic fungal strains in stored maize grains

The effect of the DCF on growth and aflatoxin B_1 production of the two aflatoxigenic fungal strains on stored maize grains was evaluated using the method as described by Afsharmanesh et al. (2018). Briefly, the 500 g surface-sterilized maize grains were soaked in 200 ml of the DCF solution and dried in a laminar airflow for 30 min. After that, the 10 ml of a spore suspension at 1×10^5 spores ml^{-1} of each aflatoxigenic fungal strain was aseptically placed in a sterile plastic bag containing 500 g grains and the whole mixed gently for 2 min (modified from Krusong et al. 2015). DMSO was served as a control. The aflatoxigenic fungi inoculated maize grains (AFIMG) samples were then stored at room temperature for 30 days and examined for evidence of the two aflatoxigenic fungal growth after spreading on PDA plates and incubation at 30 °C. Each treatment included three replicates. Aflatoxin estimation following each treatment was carried out at the same time. Extraction of aflatoxin from AFIMG employed a modification of the method described by Sidhu et al. (2009). The AFB_1 production was determined as described above.

AFB_1 degradation by the DCF and bacterial cells of S. philanthi

AFB_1 degradation by the DCF

The effect of the DCF solution at a concentration of 10% (w/v) was tested on commercial AFB_1 (Siam Inter Quality Co., Ltd., Thailand) degradation in 2-ml-Eppendorftubes a final volume of 1.0 ml according to the method of Teniola et al. (2005) with some modification. Briefly, 50 µl stock solution of AFB_1 (an initial AFB_1-concentration of 40.0 ppb) was added in 950 µl DCF solution. The mixture was incubated in the dark at 30 °C without shaking for 0, 12, 24, 36, 48, 60, 72, 84, and 96 h. DMSO plus AFB_1 served as a control. Samples after treatment were extracted with chloroform according to the Association of Official Analytical Chemists (Tosch et al. 1984) and AFB_1 concentration was determined by indirect competitive ELISA (Enzyme-linked Immunosorbent assay) using a ScreenEZ® Aflatoxin ELISA test kit (Siam Inter Quality Co., Ltd., Thailand), as described in details by Boukaew et al. (2020c). The absorbance at 450 nm (OD_{450}) was measured, using a microplate reader (M965 + MetertechInc., Taiwan). The AFB_1 concentration was estimated from OD_{450} using a Stat Fax Reader Model 321. Each treatment included three replicates. The percent degradation = {[(Control-treatment)/Control] × 100} (Branà et al. 2017) where the control was the concentration of AFB_1 in the treatment with DMSO and treatment was the concentration of AFB_1 treated with DCF solution.

AFB_1 degradation by bacterial cells of S. philanthi

AFB_1 degradation ability of bacterial cells of *S. philanthi* was tested in a test tube with a final volume of 5.0 ml according to the method of Sangare et al. (2014) with some modification. Briefly, 100 µl of a spore suspension at 1×10^7 spores ml^{-1} of *S. philanthi* was transferred to sterilized tuna condensate medium and then added with 50 µl stock solution of AFB_1 (an initial AFB_1-concentration of 40.0 ppb). The detoxification test was conducted at 30℃ for 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 days. After incubation, the cells were removed by centrifugation (8880 × g for 20 min). Sterilized tuna condensate medium was used to substitute microbial culture in the control. Samples were extracted with chloroform according to the Association of Official Analytical Chemists after treatment (Tosch et al. 1984) and AFB_1 degradation analysis was performed as described above. Each

treatment included three replicates. The percentage of AFB_1 degradation was calculated as described above.

Separation of bioactive metabolites from the DCF and testing for its efficacy against the two aflatoxigenic fungal strains using bioautography assay and identification of antifungal DCF.

Separation of bioactive metabolites from the DCF

Separation of the bioactive metabolites from the DCF solution was carried out using thin-layer chromatography (TLC) on silica gel (60 RP-18 F254S, Merck, Germany; 2 × 5 cm), with absolute methanol as the mobile phase. After that, the TLC plate was left to dry at 30 °C and sprayed with cerium sulphate (1.0%) and dried at 110 °C. The bioactive metabolite fractions were finally separated, using a bioautography assay. TLC plates were prepared in duplicate, one plate was used for bioautography assay and the other was kept for comparison.

Bioautography assay

Organic compounds, separated by TLC, were evaluated for antifungal properties. First, the TLC plate was sterilized under a UV lamp for 20 min and then placed in a PDA Petri dish, using sterile forceps. Next, it was covered by 4.0 ml of molten PDA (45 °C), containing 100 µl of spore inoculum (10^6 spore ml^{-1}) of each aflatoxigenic fungal strain. The plate was incubated at 30 °C for 48 h and the inhibition caused by the active band was observed (Azish et al. 2021).

Identification of the antifungal compounds in the DCF

The active band on the TLC plate against the two aflatoxigenic fungal strains was identified by liquid chromatography –quadrupole time of flight mass spectrometer (LC-Q-TOF MS/MS). The TLC active band was scraped and dissolved in methanol plus water (1:1) and centrifuged (at 10,000 × g for 5 min), then filtered through a 0.2 µM nylon Millipore membrane to remove silica gel and debris. The UHPLC column (Zorbax Eclipse Plus C_{18} Rapid Resolution HD 150 mm length × 2.1 mm inner-diameter, particle size 1.8 µm, Agilent) was used for the UHPLC analysis with a column temperature of 25 °C. The flow rate was 0.4 mL min^{-1}, and the mobile phase comprised 0.1% formic acid in H_2O (A) and 0.1% formic acid in acetonitrile (B). The gradient program for the mobile phase was set as follows: 0 min (A:B = 95:5), 2 min (A:B = 95:5), 40 min (A:B = 0:100), and 45 min (A:B = 0:100). The injection volume was 5 µl. The Q-TOF/MS was operated in positive and negative electrospray ionization (ESI) modes. The operating parameters were set as follows: cone voltage of 30 V, capillary voltage of 2 kV, and source temperature of 100 °C. Data were recorded in the mass-to-charge (m/z) range of 50–1200 with a scan time of 0.25 s and an interscan time of 0.02 s for 45 min. In total, 2 LC–MS chromatograms in positive or negative modes were obtained from the active band on the TLC plate and compared by mass hunter METLIN metabolite PCD (Personal Compound Database) and PCDL (Personal Compound Database and Library) version 8.

Statistical analysis

All the experiments were done in replicates (n = 3), and the data were subjected to Analysis of Variance (ANOVA), (SPSS, version 21; IBM Corp, Armonk, NY). The mean values and their significant difference were compared using Tukey's HSD (Honestly Significant Difference) test at $P < 0.05$.

Results

Efficacy of the DCF concentrations against the two aflatoxigenic fungal strains

The results showed that the inhibition on growth and AFB_1 production of the two aflatoxigenic fungal strains grown in the YES medium was related to the DCF concentrations (Table 1). AFB_1 production of *A. flavus* PSRDC-4 was 3.2 fold higher than that of *A. parasiticus* TISTR 3276 in the control (1352.18 and 423.12 ppb, respectively). By increasing the DCF concentrations from 0.2 to 7.0% (w/v), the AFB_1 production in both fungal strains was decreased up to 96.7% (from 1283.88 to 42.10 ppb) and 85.9% (from 397.52 to 56.18 ppb), respectively. In addition, the growth inhibition in both *A. flavus* PSRDC-4 and *A. parasiticus* TISTR 3276 was significantly increased from 8.23% to 93.78%, and 17.25% to 94.32%, respectively. Growth and AFB_1 production of the two fungal strains was completely inhibited (100%) at the 9.0% (w/v) DCF concentration.

Evaluation of the DCF efficacy on the growth and aflatoxin B_1 production of the two studied fungal strains in stored maize grains

The capability of the DCF to inhibit growth and AFB_1 production of the fungal strains during 28 days incubation is shown in Fig. 1. The abundant mold growth of *A. parasiticus* TISTR 3276 and *A. flavus* PSRDC-4 occurred on the control seeds in the stored maize grains but no growth occurred on the seeds treated with the DCF for 28 days of *A. parasiticus* TISTR 3276 (Fig. 1a) and 14 days of *A. flavus* PSRDC-4 (Fig. 1c). The complete inhibition of AFB_1 production on the maize grains was achieved over 28 days of *A. parasiticus*

Table 1 Effect of the DCF RL-1-178 concentrations of *S. philanthi* RL-1-178 cultivated in tuna condensate medium on the growth and aflatoxin production of *A. parasiticus* TISTR 3276 and *A. flavus* PSRDC-4 in yeast-extract sucrose (YES) broth and incubated at 30 °C for 7 days

Amount of DCF RL-1–178 solution (v/v)/45 ml YES	*A. parasiticus* TISTR 3276		*A. flavus* PSRDC-4	
	Mean of percentage mycelial inhibition ± SD	AFB_1 (ppb) production	Mean of percentage mycelial inhibition ± SD	AFB_1 (ppb) production
0 (Control)		423.12 ± 83.91^{a}		1352.18 ± 121.18^{a}
0.2	17.25 ± 1.24^{i}	397.52 ± 57.63^{b}	8.23 ± 1.50^{i}	1283.88 ± 101.25^{b}
0.4	21.88 ± 1.52^{h}	312.18 ± 11.29^{c}	16.57 ± 2.54^{h}	779.56 ± 55.68^{c}
1.0	38.31 ± 3.10^{g}	224.89 ± 18.16^{d}	33.82 ± 2.37^{g}	413.15 ± 45.20^{d}
2.0	55.00 ± 0.54^{f}	193.08 ± 9.07^{e}	51.42 ± 0.50^{f}	295.20 ± 13.97^{e}
4.0	74.62 ± 3.42^{e}	161.73 ± 16.17^{f}	78.67 ± 1.25^{e}	128.87 ± 21.48^{f}
5.0	79.80 ± 5.16^{d}	147.45 ± 36.21^{f}	85.57 ± 2.95^{d}	98.89 ± 32.48^{fg}
6.0	87.12 ± 7.87^{c}	106.63 ± 18.05^{g}	90.18 ± 3.45^{c}	69.70 ± 18.21^{gh}
7.0	94.32 ± 3.12^{b}	56.18 ± 9.12^{h}	93.78 ± 8.26^{b}	$42.10 \pm 9.62h^{j}$
8.0	98.89 ± 4.30^{a}	13.19 ± 2.13^{i}	96.43 ± 1.57^{b}	15.78 ± 3.68^{jk}
9.0	100.00 ± 0.00^{a}	0.00 ± 0.00^{i}	100.00 ± 0.00^{a}	0.00 ± 0.00^{k}
10.0	100.00 ± 0.00^{a}	0.00 ± 0.00^{i}	100.00 ± 0.00^{a}	0.00 ± 0.00^{k}

The values are means of three replicates and their standard deviation. Values followed by same letter within each column are not significantly different (ANOVA, $P < 0.05$; Tukey's HSD)

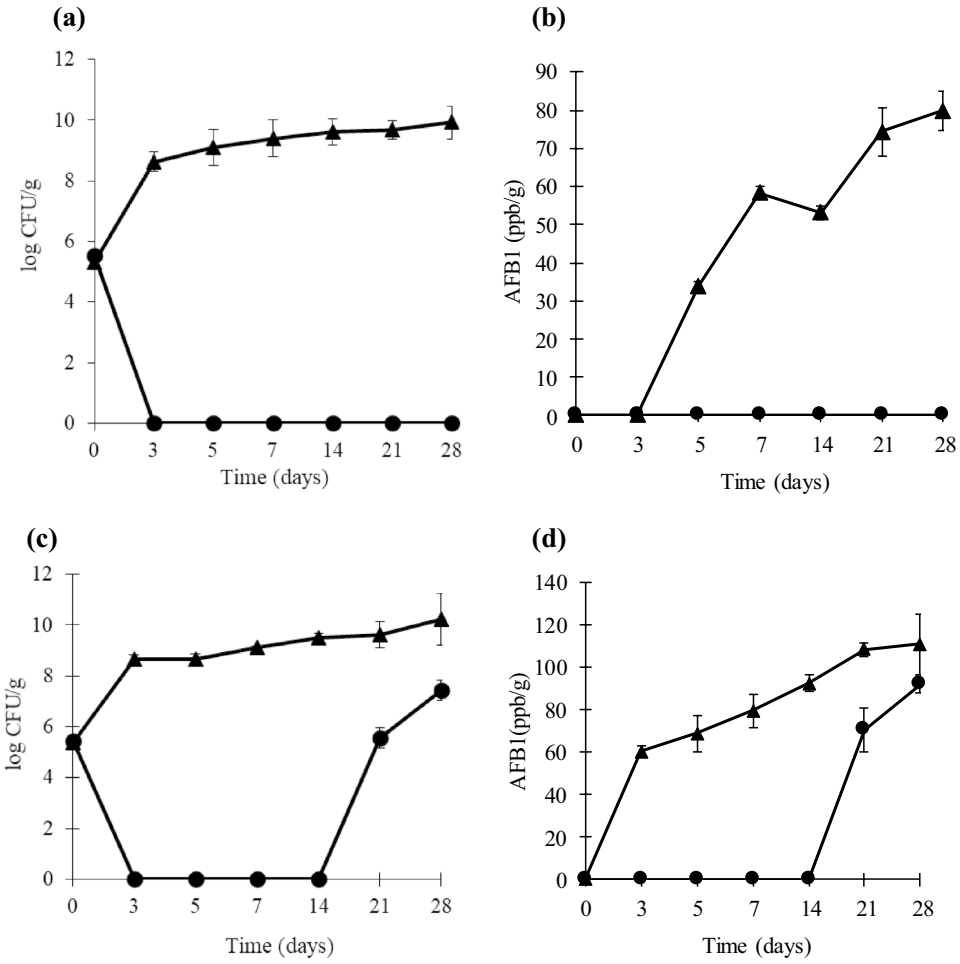

Fig. 1 Efficacy of the DCF RL-1-178 on growth and AFB_1 production of **a, b** *A. parasiticus* TISTR 3276 and **c, d** *A. flavus* PSRDC-4 on stored maize seeds. The values are means of three replicates and their standard deviation

TISTR 3276 (Fig. 1b) and 14 days of *A. flavus* PSRDC-4 (Fig. 1d).

AFB$_1$ degradation by the DCF and bacterial cells of S. philanthi RL-1–178

The capability of the DCF and bacterial cells of *S. philanthi* RL-1-178 to degrade AFB$_1$ production was shown in Fig. 2. The DCF started to degrade the AFB$_1$ at 24 h (10.93%) and significantly increased at 60 h (71.86%) and reached the highest degradation at 72 h (85.0%) (Fig. 2a). The AFB$_1$ concentration in the control tested was rather constant throughout the incubation period.

The capability of bacterial cells of *S. philanthi* RL-1-178 to degrade AFB$_1$ was presented in Fig. 2b. The AFB$_1$ degradation was first observed after 2 days (48 h) incubation (31.80%) and continuously increased to 57.50%, 77.08% degradation at 3 and 4 days, respectively, then insignificantly increased (82% to 86.89%) during 5 to 7 days, respectively. The maximum degradation of AFB$_1$ (100%) was observed after 8 days incubation. There was no obvious change in the content of AFB$_1$ in the control throughout the incubation period.

Separation and identification of bioactive metabolites from the DCF and evaluation of their efficacy against the two fungal strains using a bioautography assay

The bioactive metabolites from the DCF solution was separated in TLC by methanol (Fig. 3). The silica gel chromatograms showed five bands with the R_f of 0.12, 0.20, 0.28, 0.39, and 0.78, respectively (Fig. 3a). The strong band with $R_f = 0.78$ showed the antifungal activity against *A. parasiticus* TISTR 3276 (Fig. 3b) and *A. flavus* PSRDC-4 with positive results appeared as the clear inhibition zones around the marked fractions on the TLC plate (Fig. 3c) using bioautography assay. Identification of the bioactive metabolites using LC-Q-TOF MS/MS exhibited two compounds known as azithromycin ($C_{38}H_{72}N_2O_{12}$, $t_R = 13.0$ min) and an unknown ($t_R = 20.1$ min) (Fig. 4) with the same results based on mass ions of ESI^+ (Fig. 4a) and ESI^- (Fig. 4b) modes.

Discussion

In our previous study, the culture filtrate of *S. philanthi* RL-1-178 (grown in tuna condensate and molasses medium) at a concentration of 10.0% (v/v) with 1 h exposure time demonstrated a good efficacy to inhibit both mycelial growth and aflatoxin B$_1$ (AFB$_1$) production of *A. flavus* PSRDC-4 (Boukaew et al. 2020c). In the present study, the culture filtrate of *S. philanthi* RL-1-178 in the form of freeze dried (DCF) was tested to evaluate its degradation efficiency on the pure aflatoxin. The DCF at 9.0% w/v could effectively inhibit mycelial growth and AFB$_1$ production of *A. flavus* PSRDC-4 as well as *A. parasiticus* TISTR 3276. The effective dose (10%, v/v or 9–10%, w/v) of *S. philanthi* RL-1-178 was much lower concentration than that of *Streptomyces. globisporus* JK-1 (20%, v/v) and *S. philanthi* RM-1-138 (20%, v/v) which almost completely inhibited the growth of *Magnaporthe oryzae* (90.9%) (Li et al. 2011) and *Rhizoctonia solani* PTRRC-9 (96.0%), respectively. The high potential of the DCF to inhibit growth and aflatoxin B$_1$ production agreed with the results on mycotoxin inhibition with antifungal metabolites of *Streptomyces* strains and high degradation of AFB$_1$ as reported by Harkai et al. (2016) and Campos-Avelar et al. (2021). In addition, dioctatin A, a metabolite of *Streptomyces*, could reduce the mRNA level of *brlA* and inhibit conidiation of *A. parasiticus*, leading

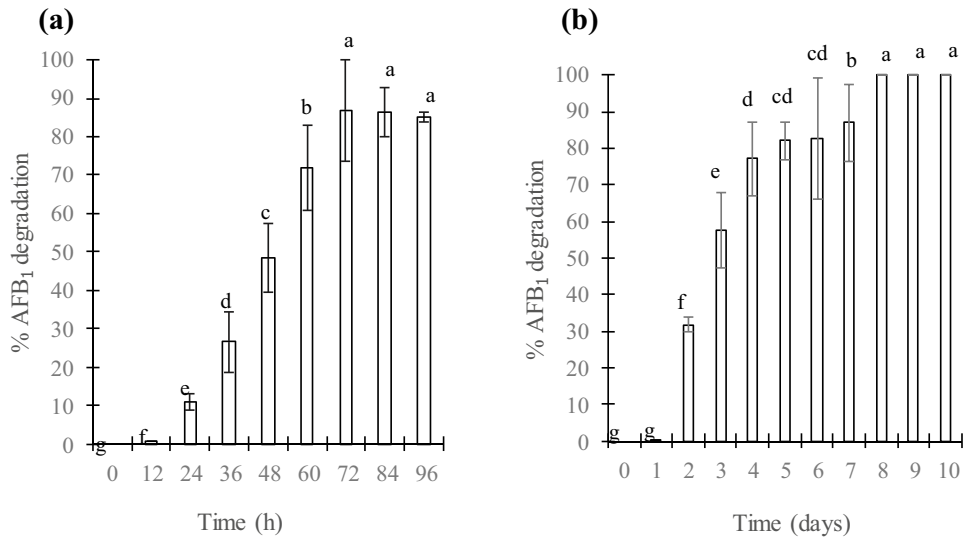

Fig. 2 Time course of in vitro AFB$_1$ degradation activity by the DCF RL-1-178 and *S. philanthi* RL-1-178 (1×10^7 spores ml^{-1}). **a** Effect of the DCF RL-1-178 on AFB$_1$ degradation activity during 96 h at 30 °C. **b** Effect of *S. philanthi* RL-1-178 (1×10^7 spores ml^{-1}) on AFB$_1$ degradation activity in tuna condensate medium during 10 days at 30 °C. The initial concentration of AFB$_1$ was 40 ppb. The values are means of three replicates and their standard deviation. Means with different letters are significantly different according to Tukey's HSD test ($P < 0.05$)

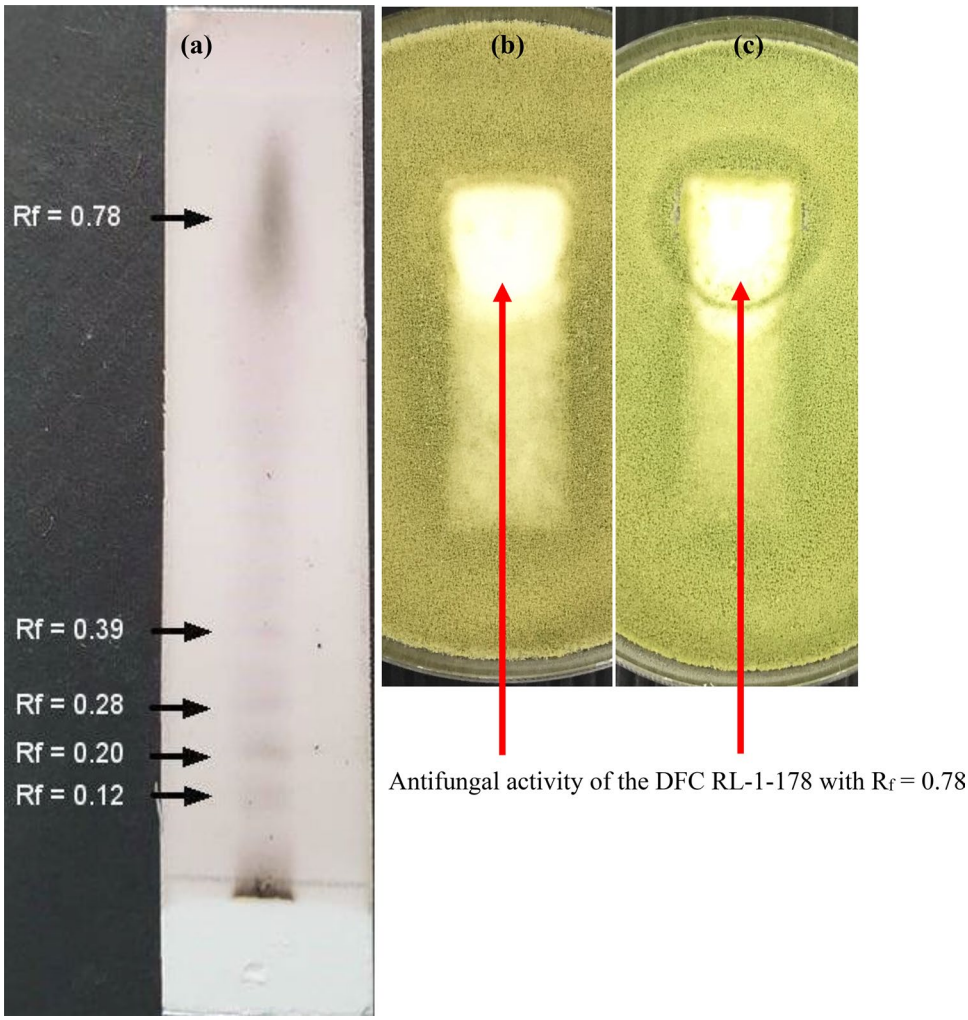

Fig. 3 Silica gel chromatograms of freeze dry bioactive compounds of *Streptomyces philanthi* RL-1-178 separated in TLC. The plates were developed in absolute methanol. Chromatograms were **a** observed after spraying with 1.0% cerium sulphate and further exposure to 110 °C **b** bioautographed against *A. parasiticus* TISTR 3276 and **c** *A. flavus* PSRDC-4

to inhibition of AFB_1 production (Yoshinari et al. 2007). Therefore, it is concluded that the substance with antifungal metabolites in the culture filtrate of *S. philanthi* RL-1-178 can inhibit conidiation of *A. parasiticus* TISTR 3276 and *A. flavus* PSRDC-4 as well as AFB_1 production. Therefore, besides measuring inhibition potential, evaluation of biological effects is an essential step in eliminating toxins.

A major problem in the storage of foods and feed stocks is spoilage and poisoning caused by fungi, *Aspergillus* species, and causes great economic losses worldwide (Magnusson et al. 2003). The effects of antifungal metabolites in the form of DCF on the growth of aflatoxigenic strains have never been reported so far. The results of the present study demonstrated for the first time that DCF has great potential in controlling postharvest disease caused by *A. parasiticus* TISTR 3276 and *A. flavus* PSRDC-4 on maize grain. High fungal growth and AFB_1 production were observed in the control treatment within 3 days. On the other hand, the application of DCF could inhibit the growth and AFB_1 production from *A. parasiticus* TISTR 3276 and *A. flavus* PSRDC-4, after 28 and 14 days of maize grain storage, respectively. Thus, it could be concluded that the antifungal metabolites of *S. philanthi* RL-1-178 could be applied in the form of DCF which shortcut the cost of purification stage. This finding suggested the simpler process to produce and apply antifungal compounds in the crude form (DCF) that able to inhibit the *Aspergillus* species. The results were in good agreement with the experiments conducted by Bressan (2009), in which the treatment of maize seeds with *Streptomyces* sp. culture filtrate reduced the development of fungi in stored seeds. Biological methods to control of post-harvest diseases in seeds are based on the use of living cells and cell-free compounds, such as *Bacillus* sp. (Ongena and Jacques 2008; Yánez-Mendizabal et al. 2012; Yánez-Mendizábal and Falconí 2018), *Trichoderma* sp. (Coşkuntuna and Özer 2008; Reddy et al. 2009; Xue et al. 2017), *Pseudomonas* sp. (Reddy et al. 2009), and *Streptomyces* sp. (Sultan and Magan 2011). One of the main factors determining the efficacy of biological control is the method of inoculation of the biological agent. This study indicated that grain inoculation with the DCF provides a significant reduction in the incidence of pathogenic seed fungi and has potential as a biological

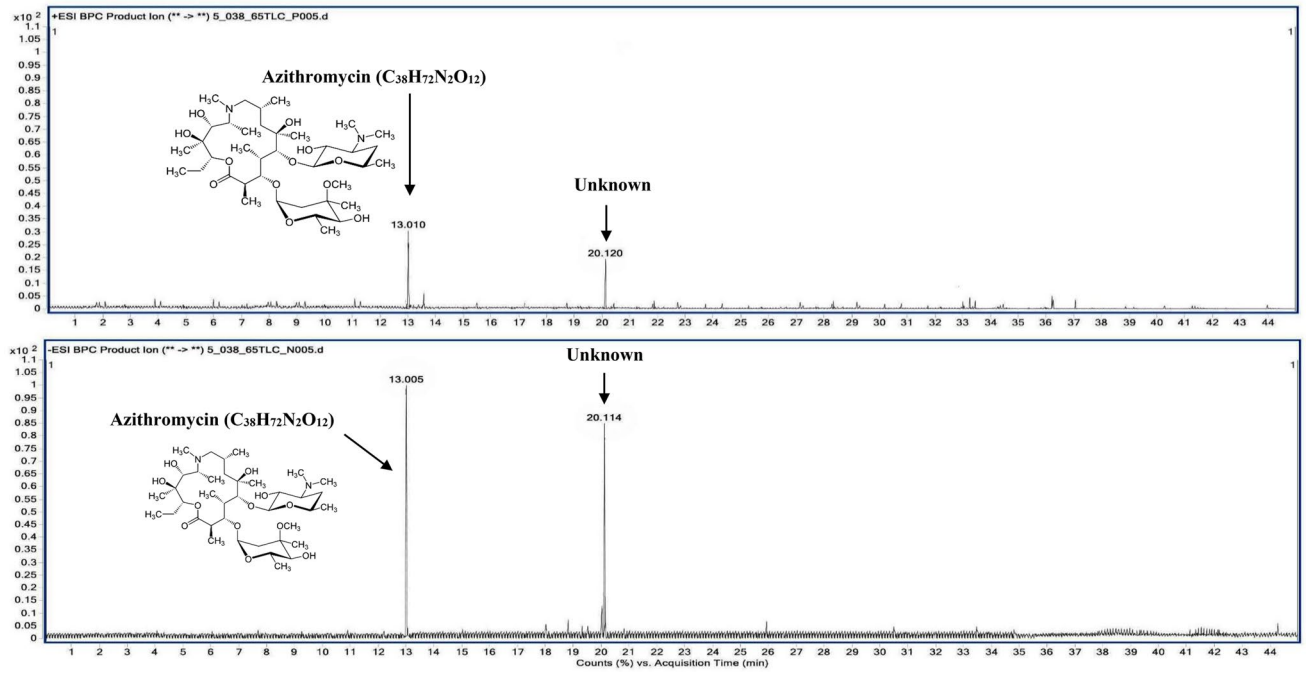

Fig. 4 Identification of bioactive compounds produced by *Streptomyces philanthi* RL-1-178 against *Aspergillus parasiticus* TISTR 3276 and *A. flavus* PSRDC-4 using liquid chromatography—quadrupole time of flight mass spectrometer (LC-Q-TOF MS/MS) based on mass ions of ESI^+ (**a**) and ESI^- (**b**) modes

control agent. Therefore, DCF was found to show significant anti-fungal growth and anti-aflatoxin B_1 production, hence, it could be explored as biopreservatives for preventing microbial deterioration and mycotoxins accumulation in food and feedstuffs during pre- and post-harvest and storage.

Streptomyces strains have been also applied in detoxification processes against mycotoxins in several ways. Some studies revealed only the antagonist effects of *Streptomyces* strains against toxin producing fungi (Harkai et al. 2016; Campos-Avelar et al. 2021). According to Verheecke et al. (2014), *Streptomyces* strains inhibited AFB_1 production in *A. flavus* by gene repression. Toxin degradation is an effective remedy for food that has been infested with toxins (Sun et al. 2023). In this study, the AFB_1-degrading capability of the DCF and bacterial cells of *S. philanthi* RL-1-178 were investigated. Our results confirmed that up to 71.86% AFB_1 was eliminated within 60 h of applying the DCF, and > 85% degradation was observed within 72 h. Results implied that the molecules present in DCF (a protein (enzyme) or proteins (enzymes) might be involved in the degradation of AFB_1. Many investigators have reported that several laccases produced by *Streptomyces* are involved in the catabolic pathways of aromatic compounds via a cascade of reactions (Park and Kim 2003; Davis and Sello 2009; Qin et al. 2021). AFB_1 is also a polyaromatic compound and could be degraded in a similar manner. Therefore, it is suggested that the DCF can cleave the lactone ring of AFB_1 and it is likely an enzymatic degradation. These results suggested that some soluble signal molecules secreted from secondary metabolites by *S. philanthi* RL-1-178 could inhibit AFB_1 biosynthesis, which was similar to the results of Harkai et al. (2016). They showed 88.34% AFB_1 degradation by the cell-free supernatant of *S. cacaoi* sub sp. *asoensis* K234 after 12 h treatment. Detoxification of AFB_1 by cell-free supernatant obtained from many antagonistic bacterial strains, such as *R. erythropolis* and *Mycobacterium fluoranthenivorans* sp. nov. DSM44556T (Teniola et al. 2005), *Pseudomonas* sp. (Sangare et al. 2014), *B. licheniformis* CFR1 (Rao et al. 2017), *B. subtilis* (Xia et al. 2017; Suresh et al. 2020), *B. velezensis* DY3108 (Shu et al. 2018), and *B. megaterium* (Wang et al. 2021) has previously been reported. The majority of the studied bacterial cells of *Streptomyces* isolates were able to degrade AFB_1, either in solid or liquid media. This is in agreement with the findings of Campos-Avelar et al. (2021) and Harkai et al. (2016) whose degradation assays with *Streptomyces* strains proved that all of them were able to significantly degrade AFB_1. In general, aflatoxin degradation by *Streptomyces* has been demonstrated by several authors (Verheecke et al. 2015; Harkai et al. 2016; Avelar et al. 2021). Herein, the AFB_1 degrading potential value (100%) of *S. philanthi* RL-1-178 was similar to those of *Streptomyces. lividans* and *Streptomyces. aureofaciens* (Eshelli et al. 2015) but higher than the results of other *Streptomyces* species. These included the *Streptomyces*

isolate MYC (31%) (Campos-Avelar et al. 2021), *S. cacaoi* subsp. *asoensis* K234 (88.33%), *Streptomyces. sanglieri* K139 (61.43%), *Streptomyces. luteogriseus* K144 (79.93%), *Streptomyces. rimosus* K145 (79.93%), and *Streptomyces. cinereoruber* K236 (58.52%) (Harkai et al. 2016). Thus, different AFB_1 degradation activities among various *Streptomyces* species were the result of different levels of active compounds in cell-free supernatant which are responsible for the diminished AFB_1. From the experiments conducted, the DCF and bacterial cells of *S. philanthi* were capable to degrade AFB_1 by 85.0% and 100% for 72 h and 8 days, respectively. This confirmed the higher efficacy of the DCF over the cells. This is in agreement with the findings that the cell-free supernatant was predominantly attributed in the AFB_1 degrading activity than bacterial cells (Xia et al. 2017; Shu et al. 2018). Dioctatin A (DotA), a metabolite of *Streptomyces*, inhibited production of norsolorinic acid which is an early biosynthetic intermediate of aflatoxin produced by *A. parasiticus* (Yoshinari et al. 2007). In addition, it also strongly reduced the mRNA levels of genes responsible for aflatoxin biosynthetic enzymes and the mRNA level of *aflR* encoding a key regulator protein for aflatoxin biosynthesis. There is limited evidence on the capability of *Streptomyces* sp. on decrease of AFB_1-contents (Sakuda et al. 1996; Zucchi et al. 2008; Harkai et al. 2016), whereas several reports demonstrated that some strains of *Streptomyces* sp. could inhibit the mycelial growth and aflatoxin production of *A. flavus* (Sultan and Magan 2011; Caceres et al. 2018; Shakeel et al. 2018; Boukaew et al. 2020b,c; Campos-Avelar et al. 2021) and *A. parasiticus* (Boukaew et al. 2020b,c). However, the present study exhibited that the DCF and bacterial cells of *S. philanthi* RL-1-178 had a high potential for degrading AFB_1. The DCF had highly inhibitory effects on growth and AFB_1 production of *A. parasiticus* TISTR 3276 and *A. flavus* PSRDC-4 both in the in vitro and on maize grains.

Separation and localization of the bioactive compounds by TLC on silica gel and bioautography were again proved the presence of antifungal compounds. Based on the TLC results, the DCF with $R_f = 0.78$ exhibited antifungal activities on both aflatoxin-producing fungi, as confirmed by the bioautography assay. The result is similar to the observation of Azish et al. (2021) that the partial purification of the antifungal metabolites of *Streptomyces. libani* on TLC with $R_f = 0.88$ exhibited anti-*Aspergillus. fumigatus* activities while the bioactive compound of *Streptomyces. albidoflavus* 321.2 with $R_f = 0.85$ showed antifungal activity against *Aspergillus. niger* (Eshelli et al. 2015). *Streptomyces* produced antimicrobial compounds that showed R_f values ranging from 0.40 to 0.78 in TLC analysis, which confirmed the production of polyene nature of compounds (Selvakumar et al. 2010). The active band was separated as white amorphous powder, which was identified as azithromycin ($C_{38}H_{72}N_2O_{12}$, $t_R = 13.0$ min) and an unknown substance ($t_R = 20.1$ min) based on the mass ions of ESI^+ and ESI^-modes. Azithromycin, a second generation macrolide, broad-spectrum antifungal substance against *Aspergillus* sp. (Nguyen et al. 1997; Guo et al. 2018) and *Fusarium solani* (Guo et al. 2018) and antibacterial substance against *Bordetella pertussis* and *Legionella* sp. (Parnham et al. 2014), has received increasing attention in recent years because of additional effects on host-defense reactions and chronic human diseases. It also has activity against *Mycoplasma pneumoniae*, *Treponema pallidum*, *Chlamydia* sp., and *Mycobacterium avium* complex (Parnham et al. 2014). It was reported that azithromycin exerted its antifungal activity against *Aspergillus* species by inhibiting mitochondrial and cytoplasmic protein synthesis (Nguyen et al. 1997). The DCF revealed two compounds which were identified as azithromycin and an unknown substance that possessed antifungal activity. Therefore, it could be concluded that the antifungal activity of the integral DFC may be attributable to two compounds resulting from independence or synergistic effect.

In conclusion, the antifungal and anti-mycotoxigenic capabilities of the DCF RL-1-178 both in vitro and on maize grains were evaluated. The DCF was applied to inhibit fungal growth and aflatoxin production, both in vivo and in vitro. These results indicated that the antifungal metabolites of *S. philanthi* RL-1-178 could be considered as potential biocontrol agents to combat toxigenic fungal growth and subsequent aflatoxin contamination of maize and other agricultural crops in practice.

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Declarations

Conflict of interest The authors have not disclosed any competing interests.

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