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Protein-mediated degradation of aflatoxin B_1 by *Pseudomonas putida*

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Abstract

Degradation or the removal of aflatoxin B_1 from agriculture commodities is very important because of its acute toxicity and economic loss due to rejection of about 25% contaminated agri produce. The present study aimed at using *Pseudomonas putida* for the aflatoxin B_1 (AFB_1) degradation and to understand the mechanism involved. AFB_1 degradation was studied with *P. putida* culture, culture supernatant, cell lysate, cell lysate in the presence of protease inhibitor, and heat-inactivated cell lysate. The remaining AFB_1 was qualitatively and quantitatively measured by thin-layer chromatography and HPLC with a UV detector. *P. putida* culture and culture supernatant showed 80% reduction in AFB_1 within 24 h of incubation. Cell lysate and the lysate in the presence of protease inhibitor showed the same reduction in 6 and 4 h respectively. The protease-inhibited lysate showed greater thermostability, broad pH range, and tolerance to some of the solvents and detergents in terms of aflatoxin B_1 degrading activity. The heat-inactivated lysate showed only 20% reduction in 24 h of incubation indicating loss of activity on heating. As cell-free supernatant and cell lysate are capable of reducing AFB_1 effectively, actively growing cells are not necessary for degradation. The active principle for degradation might be proteinaceous; therefore, heat-inactivated lysate is ineffective for reducing the AFB_1. These results showed that degradation of aflatoxin B_1 by *P. putida* might be an enzymatic process and could be used in a broad range of conditions.

Keywords Aflatoxin B_1 · Bioremediation · Food safety · Industrial applications · *Pseudomonas putida*

Introduction

The Food and Agriculture Organization (FAO) estimated that 25% of the world's food crops are affected by mycotoxins every year. In Indonesia, Thailand, and the Philippines, 5% of the peanuts and maize were discarded due to fungal contamination [1]. There are reports on the annual loss of nearly $500 and $750 million in the USA and Africa respectively, due to aflatoxin-contaminated agricultural crops [2, 3]. In 2016, the corn industry in the USA estimated a loss of US$ 52.1 million to 1.68 billion [4].

Due to its notable and harmful impact on health and economic trade, aflatoxin B_1 (AFB_1) has attracted worldwide awareness. Aflatoxin B_1 is known to be hepatotoxic, carcinogenic, mutagenic, teratogenic, and immunosuppressive to both humans and animals [5]. AFB_1 is listed as the group I carcinogen by the International Agency for Research on Cancer [6]. Various strategies have been reviewed for removal and detoxification of mycotoxin which includes physical (thermal inactivation, photochemical irradiation, or gamma irradiation) and chemical (with acids, alkalis, aldehydes, oxidizing agents, and gases like chlorine, sulfur dioxide, ozone, and ammonia) methods [7–9]. None of these methodologies completely fulfill the desired efficacy, safety, and nutrient retention. Several reports are available where microorganisms mainly bacteria have been used to either degrade mycotoxins or reduce their bioavailability [10–15]. Studies for aflatoxin removal by different microorganisms reported that it can bind to the bacterial cell wall in a rapid process [16, 17]. Enzymatic degradation of AFB_1 by *Rhodococcus erythropolis* and *Mycobacterium fluoranthenivorans* sp. was reported [18–20]. In *R. erythropolis* culture, 83% AFB_1 degradation was noted after 48 h of incubation, and in cell-free culture it was undetectable after 8 h of incubation, as described by Alberts et al. [20]. Similarly, Guan et al. [21] reported more efficient degradation of AFB_1 by the culture supernatant of

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Stenotrophomonas maltophilia than the viable cells and cell extracts. *Pseudomonas* is part of a large, ubiquitous, and heterogeneous group of microorganisms commonly referred to as pseudomonads. *P. putida* is a gram-negative, rods with polar flagella; they are chemo-organotrophs, non-fermentative type of respiratory metabolism and are usually catalase- and oxidase-positive [22]. *P. putida* is widely known to play an efficient role in the acidification of organic matter produced in nature, industrial products, and the decomposition of sewage. Different types of plasmids like NAH, SAL, CAT, CAM, TOL, and PWWO were noted to be present in *P. putida* and capable to degrade aromatic hydrocarbons like napthalene, salicylate, camphor, toluene, methyl benzoate, and para-nitrophenol [23–25]. There are several reports on enzymes like laccases, peroxidases, oxidases, and reductases involved in the aflatoxin degradation process [18, 26–28]. Exploration of the degradative way directed that each microorganism has a dissimilar way of degrading AFB_1. Samuel et al. [29] have studied the AFB_1 degradation by *P. putida* strain 2445. It has been proposed that the lactone ring of AFB_1 opened in the presence of *P. putida*, which resulted in the transformation of AFB_1 into structurally different non-fluorescent compounds AFD1 and AFD2 [29]. In the present study, we are mainly concentrating on finding the active constituent responsible for AFB_1 degradation and its stability in various physical conditions and presence of chemicals. For this purpose, AFB_1 degradation was performed in the presence of *P. putida* viable cells, culture supernatant, cell lysate, and cell lysate in the presence of protease inhibitor (PMSF) and heated lysate. The stability of the most active constituent was studied by treating at different temperatures and pH and in the presence of organic solvents or detergents. The amount of degraded AFB_1 was estimated using analytical methods like thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC).

Materials and methods

Microorganisms and culture conditions

A toxigenic strain of *Aspergillus flavus* (PP3) was isolated from peanut in our laboratory (VIT, Vellore, India) and identified as *A. flavus* link 1809 based on partial DNA sequence (545 bases) similarity with NCBI sequence accession Q14704. The culture was maintained on potato dextrose agar (PDA) medium slants at 4 °C throughout this study. Aflatoxin B_1 (AFB_1) was produced from toxigenic strain of *A. flavus* spore suspension (10^6 spores ml^{-1}), and inoculated in 20 g of autoclaved rice with 22 to 23% of moisture content maintained at the time of fungal inoculation. Flasks were incubated at 28 °C for 5 days in the laboratory and purified as previously described by Aiko and Mehta [30]. *P. putida* strains (MTCC 2445 and 1072) used in this study were procured from the Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India, and maintained on nutrient agar medium (HiMedia Laboratories Pvt. Ltd., India) at 4 °C. Further for experimental studies, *P. putida* strains (MTCC 2445, 1072) were cultured in mineral salt glucose medium (MSG) and incubated in a shaker at 120 rev min^{-1} at 37 °C temperature for different time intervals [31].

Growth measurement

For the growth studies, both *P. putida* strains (MTCC 2445 and 1072) were cultured for 22 h in 100-ml conical flasks containing sterile MSG broth. Aliquots (100 μl) of the respective bacterial culture of 0.5 OD_{600} corresponding to $1.86 \times 10^6 \pm 0.03$ CFU ml^{-1} were inoculated in 10 ml sterile MSG containing AFB_1 0.2 μg ml^{-1} or without AFB_1. Flasks were incubated for 72 h at 37 °C and 120 rev min^{-1} shaking. The growth of the *P. putida* strains was determined taking the OD_{600} in a spectrophotometer (UV-Visible 1800, Shimadzu, Japan) at an interval of 6 h. The dynamics of the bacterial growth were studied by plotting the optical density (OD_{600}) versus incubation time (h). The bacterial growth rate constant (k) was calculated using this formula:

$$k = \frac{(\log N_t - \log N_0)}{(0.301\ t)}$$

N_0 Initial Number of cells
N_t Number of cells at time t
t Generation time

Degradation studies

A degradation study of AFB_1 has done in MSG medium for 24 h following the method given by Samuel et al. [29]. One milliliter of *P. putida* culture (0.5 OD_{600}) corresponding to $1.86 \times 10^6 \pm 0.03$ CFU ml^{-1} was inoculated with 9 ml of MSG medium in 100-ml conical flasks and incubated at 37 °C for a period of 24 h, in an incubator shaker at 120 rev min^{-1}. At the end of the incubation period, the culture was centrifuged at 3000 rpm (Eppendorf centrifuge 5804 R, rotor A-4-44) for 10 min at 4 °C to separate the bacterial cells and supernatant. Further, the supernatant was filtered through a 0.22-micrometer (μm) filtration membrane to remove the bacterial cells. Nutrient agar plating up to 24 h was used to confirm that supernatant was free from cells. Cell-free supernatant was stored at − 80 °C for further studies. The cell pellets were then washed with 5 ml phosphate-buffered saline (PBS) pH 7.4, resuspended in the same buffer before lysis.

Cell lysis was carried out in the presence and absence of 0.5 mmol l^{-1} PMSF (phenylmethylsulfonyl fluoride) using a sonicator at 4 °C, 4 pulse cycles with each lasting for 10 min [19]. After the sonication process, lysed cells were again centrifuged at 12,000 rpm for 20 min at 4 °C (Eppendorf centrifuge 5804 R, rotor FA-45-6-30). The lysate was filtered using a 0.22-μm pore syringe filter (Nupore Filtration Systems, India) and stored at − 80 °C for additional use. To determine whether the effect of the lysate on the AFB_1 degradation can be attributed to protein activity, it was subjected to heat inactivation treatment, following the method described by Guan et al. [21]. Lysate was taken from − 80 °C and thaw under the running water. Twenty milliliters liquid lysate (with PMSF) was taken into a 100-ml conical flask and heated in a water bath at 80 °C for 10 min. The stock of AFB_1 was prepared at a concentration of 200 μg ml^{-1} in dimethyl sulfoxide (DMSO). The final concentration of AFB_1 was made to 0.2 μg ml^{-1} of the medium. Seven experimental sets were prepared; each set contained different samples: set I: MSG media, set II: MSG + AFB_1, set III: MSG + culture + AFB_1, set IV: MSG + culture supernatant + AFB_1, set V: MSG + cell lysate + AFB_1, set VI: MSG + PMSF lysate (cell lysis in presence of PMSF) + AFB_1, set VII: MSG + heat-inactivated lysate (80 °C for 10 min) + AFB_1 as given in Table 1 and were incubated in an orbital shaker at 37 °C over a period of 24 h at 120 rev min^{-1}. After each incubation period, the samples were extracted with an equal volume of chloroform in a separating funnel. Extraction was done twice; the extracts were pooled and concentrated to a volume of 1 ml. These samples were then assayed analytically and the remaining AFB_1 in the sample was analyzed qualitatively and quantitatively. Further for better characterization, stability tests of protease-inhibited lysate were performed against temperatures, pH ranges, solvents, or detergents.

Stability of lysate + PMSF (set VI) on AFB_1 degradation in different conditions

Temperature

The cell lysate (set VI) was kept for 24 h at 37 °C or 50 °C temperature and then, the AFB_1 degradation activity was assessed. MSG broth (pH 7.0) treated in the same manner as the test has been used as control.

pH

The pH of the cell lysate (set VI) was adjusted to 3–11 pH range and kept for 24 h at 37 °C and then, the activity was assessed. In the pH tests, phosphate-citrate buffer was used to adjust pH 3.0–7.0 and glycine-NaOH to pH 9 to 11. MSG broth treated in the same manner as the test has been used as control.

Solvents

Lysate activity was assessed in the presence of chloroform ($CHCl_3$), dimethyl sulfoxide (DMSO), isopropanol (C_3H_8O), and benzene (C_6H_6) solvents by incubating lysate with 0.1% and 0.5% v/v solvent for 24 h at 37 °C.

Detergents

Lysate activity was assessed in the presence of common detergents SDS, Tween-20, Tween-80, and Triton X-100 by incubating lysate with 1%, 5%, and 10% v/v detergent for 24 h at 37 °C.

After the incubation at different temperatures and pH or with organic solvents and detergents, all the test and control lysates were further incubated with AFB_1 (0.2 μg ml^{-1}) for 8 h at 37 °C, 120 rev min^{-1}. Samples were extracted with chloroform and analyzed for AFB_1.

Effect of temperature on AFB_1 degrading activity of set VI

Additionally, the temperature versus activity profile of set VI to degrade AFB_1 was also assessed in the range of 20–100 °C.

Measurement

AFB_1 measurement

Qualitative analysis of AFB_1 using thin-layer chromatography (TLC) was performed along with a standard AFB_1 (Supelco, Bellefonte, PA, USA). Five microliters of the extract was applied to activated TLC plate (Silica gel 60, MachereyeNagel, Germany); chloroform:acetone (85:15) was used as the solvent system. Quantitative analysis with HPLC (HPLC, waters 1525 with UV detector) was done using the mobile phase water:acetonitrile:methanol (60:20:20, v/v/v) isocratic at a flow rate of 1 ml min^{-1} and an injection volume of 20 μl, measured by a UV detector at 360 nm. Standard AFB_1 was used to check the enactment of the technique. The deviation in the peak area and the retention time was within the 5% error limit in terms of accuracy. The detection limit (LOD) was 6 ng ml^{-1} under the experimental conditions [30]. All the experiments were performed in triplicates. AFB_1 concentration in the test samples using HPLC was calculated by this formula:

$$\text{Concentration unknown} = (\text{Area unknown}/\text{Area known})\ \text{Concentration known.}$$

Table 1 Samples of *P. putida* strain (MTCC 2445) were prepared in different conditions for experimental studies

Set	Sample	Condition
I	Blank	MSG media
II	Control	MSG + AFB_1
III	Test 1	MSG + culture+AFB_1
IV	Test 2	MSG + culture supernatant + AFB_1
V	Test 3	MSG + cell lysate + AFB_1
VI	Test 4	MSG + PMSF lysate (cell lysis in presence of PMSF) + AFB_1
VII	Test 5	MSG + heat-inactivated lysate (80 °C for 10 min) + AFB_1

The % degradation in the test sample is calculated by this formula:

$$\%\text{Degradation} = \frac{\text{Amount in control } \mu g/ml - \text{Amount in test } (\mu g/ml)}{\text{Amount in control } (\mu g/ml)} \times 100$$

Protein estimation

Protein concentration of the lysate of *P. putida* in the presence or absence of PMSF was determined using the Bradford method [32]. A standard curve was prepared using known concentrations in the range of 5–50 μg ml^{-1} of bovine serum albumin (BSA). Total protein concentrations of the crude extracts were subsequently extrapolated from the standard curve.

Statistical analysis

The AFB_1 percent degradation, protein concentration data, stability of set VI on AFB_1 degradation in different conditions, and effect of temperature on AFB_1 degrading activity of set VI obtained were analyzed by ANOVA using Microsoft Excel 2010 to know variation among and between test sets. A significant *F*-test at $p < 0.05$ level of probability was then reported. The results represent the average of triplicate and are expressed as mean ± standard deviation.

Results

Purification of AFB_1

The AFB_1 used in the present study was produced and purified in the laboratory from the toxigenic strain of *A. flavus*. The purity was confirmed by spiking with standard AFB_1 on TLC and HPLC showing a single peak at 7.29-min retention time. Yield was 112.22 ± 0.94 μg g^{-1} of rice.

Growth of *P. putida* strains (MTCC 2445 and 1072) in presence of AFB_1

The growth rate of *P. putida* strains (MTCC 2445 and 1072) was calculated from the optical density measured at different time intervals. The generation time varied for both the strains. The growth rate constant obtained for *P. putida* strains (MTCC 2445 and 1072) is 0.23 h^{-1} and 0.34 h^{-1} respectively. Figure 1 shows that *P. putida* in MSG media grew fast after a lag phase of 12 h and remains in exponential phase up to 24 h. In the presence of AFB_1 (0.2 μg ml^{-1}), the growth of both strains was retarded. The growth rate constant obtained for test samples (*P. putida* + AFB_1) is 0.09 h^{-1} and 0.08 h^{-1} in the same generation time of 8.0 h for strains (MTCC 2445 and 1072) respectively. Lag phase extended up to 20 h and remained in exponential phase up to 55 h. However, while nearing the end of incubation (72 h), the growth was almost similar to that of control. These results show that AFB_1 is not lethal to the *P. putida*. However, it retarded the growth of bacteria due to the toxic nature of AFB_1.

Degradation of AFB_1 by *P. putida* strains (MTCC 2445 and 1072)

P. putida cells harvested during the exponential growth phase (log phase) and incubated with AFB_1 showed a reduction in AFB_1 content with time. The residual AFB_1 was calculated from the standard curve plotted between absorbance at 360 nm and concentration in the range of 0.25 to 1.5 μg ml^{-1}. A gradual reduction was observed in AFB_1 content by both *P. putida* strains (MTCC 2445 and 1072) (set III). After 24 h of incubation, more than 80% AFB_1 was degraded (Fig. 2). No significant difference was noted in the degradation of AFB_1 by both *P. putida* strains (MTCC 2445 and 1072); henceforth, work is carried out using only *P. putida* strain (MTCC 2445). To know if the AFB_1 degradation is carried out by actively dividing cells or the enzymes produced during growth, the experiments were performed by incubating AFB_1 with culture supernatant (without any live cells) and cell lysate.

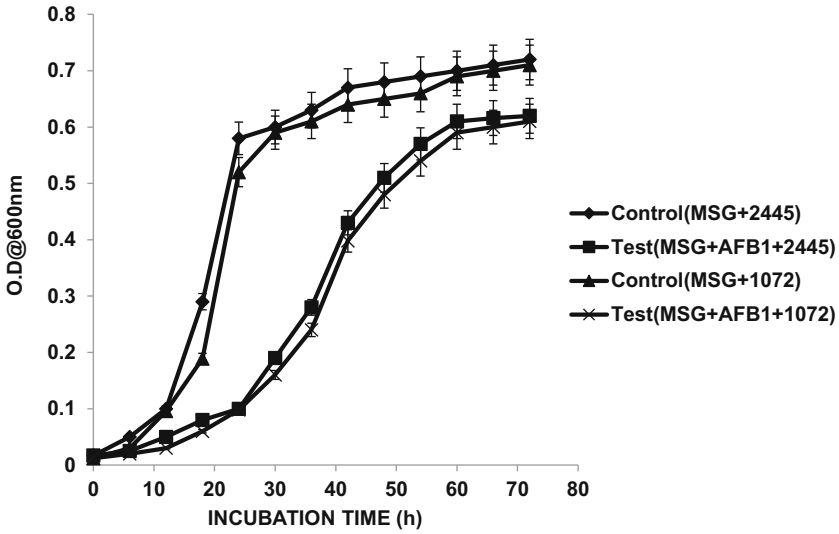

Fig. 1 Growth curve of *P. putida* strains (MTCC 2445 and 1072) in presence and absence of AFB_1. Culture conditions: culture medium—minimal salt, glucose; incubation temperature—37 °C; pH—7; incubation time—72 h. Optical density measured at 600 nm. The values are means of triplicates and their standard errors shown as a bar

AFB_1 degradation by culture supernatant and lysates

Culture supernatant or spent medium of the bacterial strain was explored for their propensity to degrade AFB_1. The untreated sample set II remains unchanged throughout the experimental period of 24 h. More than 90% of AFB_1 degradation was observed by set IV (Fig. 3a). After 24 h of incubation time, sets V and VI have shown 94% and 100% AFB_1 degradation respectively. No detectable amount of AFB_1 was noted in set VI after 24 h of incubation time. Set VI in the presence of PMSF showed 75% AFB_1 degradation within 2 h of incubation time. While sets III, IV, V, and VII showed 10%, 14%, 58%, and 5% AFB_1 degradation in the same incubation time. As shown in (Fig. 3a), the AFB_1 degradation is much faster with the lysate in presence of PMSF than without PMSF as 70% degradation could be achieved within 1 h of incubation time while the lysate took 4 h to degrade similar amount of AFB_1. Heated lysate lost the AFB_1 degrading capability as only 20% degradation was noted after 24-h incubation, indicating that after heating protein might have been denatured and hence lost the activity. These results are well correlated with the disappearance of fluorescent spots on TLC plates (Fig. 3b). The minimum detection limit of AFB_1 by HPLC was observed as 6 ng ml^{-1} [30]. The results from TLC and HPLC analyses confirmed that AFB_1 was significantly degraded by *P. putida.* The protein content of cell lysate as measured by the Bradford assay was found higher in presence of protease inhibitor, the PMSF (22 μg ml^{-1}), compared with cell lysate (18 μg ml^{-1}) in absence of PMSF (Fig. 4) correlating higher AFB_1 degradation (Fig. 3). This might be due to protein degradation by the protease released during cell lysis. Results indicated that the active molecule responsible for the degradation of AFB_1 by *P. putida* may be proteinaceous and the degradation of AFB_1 might be the enzymatic reaction.

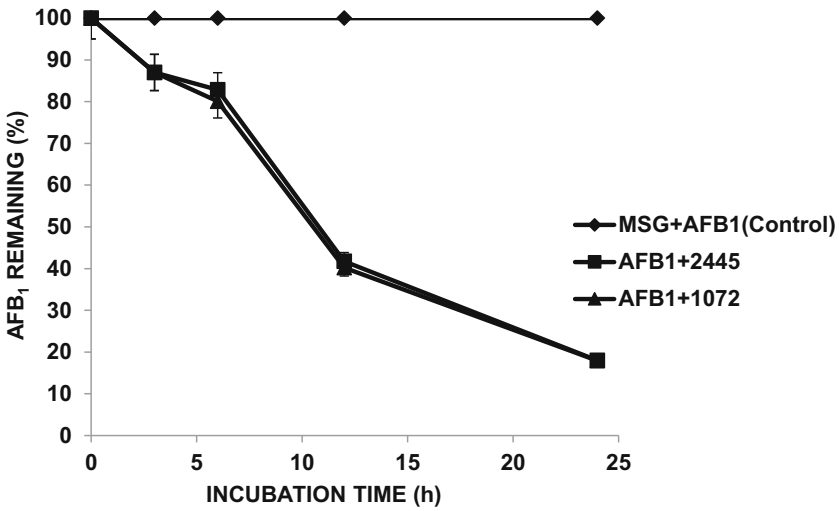

Fig. 2 AFB_1 degradation by *P. putida* strains (MTCC 2445 and 1072) at different incubation times. Culture conditions: culture medium—minimal salt glucose; incubation temperature—37 °C; pH—7. The values are means of triplicates and their standard errors shown as a bar

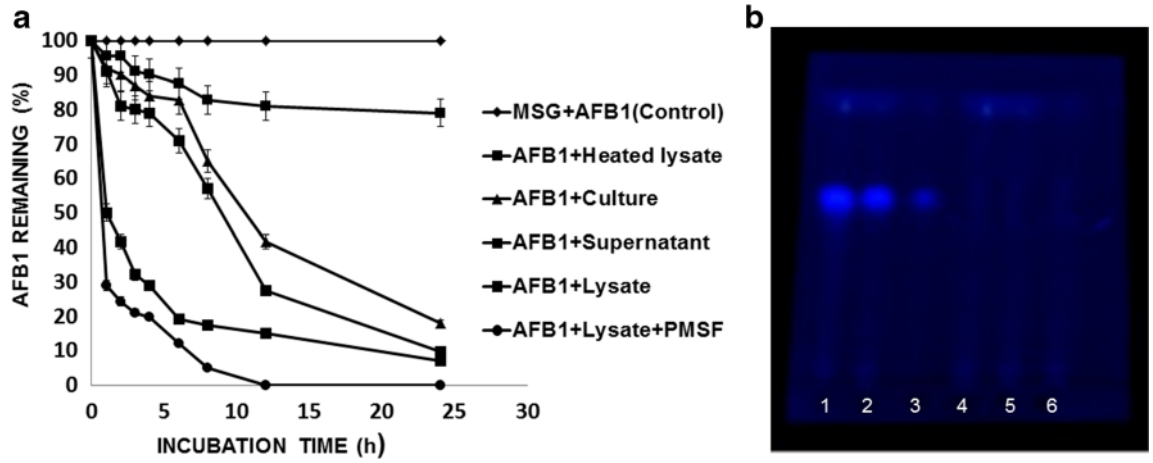

Fig. 3 **a** AFB_1 degradation in the presence of *P. putida* strain (MTCC 2445) culture, culture supernatant and cell lysate over a period of 24 h at 37 °C. The values are means of triplicates and their standard errors shown as a bar. **b** Silica gel thin-layer chromatogram of aflatoxin B_1 after incubation for 24 h with (1) standard aflatoxin B_1; (2) *P. putida* lysate heated at 80 °C for 10 min; (3) *P. putida* culture; (4) *P. putida* culture supernatant; (5) *P. putida* cell lysate; and (6) cell lysate prepared in the presence of protease inhibitor (PMSF). The solvent system used is chloroform:acetone (85:15)

Tolerance/stability profile of *P. putida* (lysate + PMSF, set VI) on AFB_1 degradation

The stability of set VI after exposure to various temperatures, pH, solvents, or detergents were studied by assaying the AFB_1 degradation activity. The cell lysate: set VI was incubated for 24 h at 37 and 50 °C before the addition of AFB_1 and the degradation of AFB_1 was observed at 37 °C, pH 7 for 8 h. Results showed 94% AFB_1 degradation in the sample kept at 37 °C which is similar to the control (94.6%) without incubation, indicating that the cell lysate is stable and can be kept at room temperature. Incubation at 50 °C enhanced the activity showing the complete degradation of AFB_1. Table 2 shows that the lysate is more active in the alkaline pH range of 7.0 to 11.0 as the highest and 94% AFB_1 degradation was obtained at pH 7.0, 85% at pH 9, and 58% at pH 11. In acidic pH 3 and 5, AFB_1 degradation was very less. These results show that the lysate is active in neutral or alkaline conditions, but not in acidic condition. In the presence of solvents, AFB_1 degradation ability of the lysate significantly decreased (Table 2). Among the solvents used, DMSO had least effect and benzene had the highest effect showing 79% and 28% degradation respectively. Presence of chloroform also inhibited the degradation. The effect was more profound in higher concentration (0.5%) of solvent in comparison to lower concentration (0.1%) used in the experiment. Among the detergents tested, anionic detergent (sodium dodecyl sulphate, SDS) affected the degradability of the lysate most as only 13% degradation was noted. While in the presence of non-ionic detergents (Tween 20, Tween 80, and Triton X-100), more than 40% AFB_1 was degraded. Set VI was highly stable in the presence of Triton X-100 as even 10% v/v showed more than 75% degradation. These results suggest that the *P. putida* lysate is stable in various conditions and can be stored and used in different matrices for AFB_1 degradation.

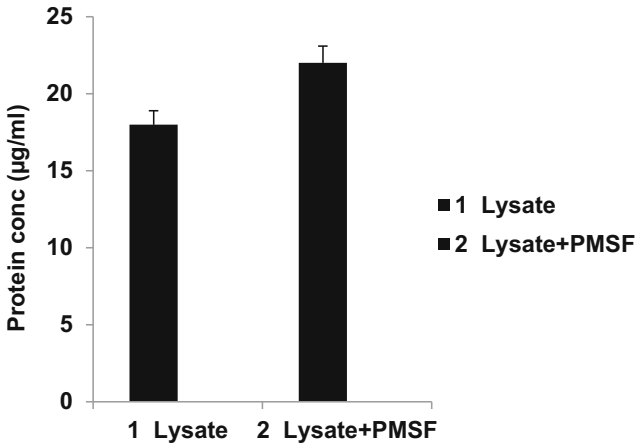

Fig. 4 Protein estimation by Bradford method in cell lysate in the absence or presence of PMSF. The values are means of triplicates and their standard errors shown as a bar. *$p \leq 0.05$

Effect of temperature on AFB_1 degradation activity of *P. putida* (lysate + PMSF; set VI)

Set VI: the cell lysate showed the activity in a broad range of temperature from 30 to 70 °C. Surprisingly, the activity increase with increasing temperature and highest degradation (90%) was noted at 70 °C within 1 h (Table 3). At 100 °C, lysate lost its activity and negligible degradation was observed.

Table 2 Stability/tolerance profile of *P. putida* lysate prepared in the presence of protease inhibitor (PMSF) in terms of AFB_1 degrading activity at different temperatures and pH, in the presence of organic solvents or detergents

Sample set VI[(1)] with treatment[(2)]	AFB_1 degradation (%) within 8 h	Sample set VI[(1)] with treatment[(2)]	AFB_1 degradation (%) within 8 h		
Temperature (° C)		Solvents	0.1% (v/v)	0.5% (v/v)	
37	94.10 ± 0.03	$CHCl_3$	50.00 ± 0.01	35.30 ± 0.00	
50	100.00 ± 0.00	DMSO	82.20 ± 0.02	79.20 ± 0.01	
		C_3H_8O	61.60 ± 0.01	55.30 ± 0.02	
		C_6H_6	48.90 ± 0.02	28.30 ± 0.01	
pH		Detergents	1% (v/v)	5% (v/v)	10% (v/v)
3	15.00 ± 0.01	SDS	70.50 ± 0.01	47.60 ± 0.02	13.80 ± 0.01
5	29.10 ± 0.01	Tween-20	88.00 ± 0.01	58.30 ± 0.02	47.60 ± 0.02
7	94.60 ± 0.02	Tween-80	76.00 ± 0.02	55.00 ± 0.01	40.20 ± 0.01
9	88.00 ± 0.02	Triton X-100	88.30 ± 0.01	80.00 ± 0.01	75.30 ± 0.01
11	58.27 ± 0.02				

[(1)] Set VI: *P. putida* lysate in the presence of PMSF

[(2)] Treatment of set VI in respective conditions for 24 h

Discussion

The study shows *P. putida* is able to grow in the presence of AFB_1 though the exponential growth phase got delayed; it is not lethal [29]. Good degradation was also noted of added AFB_1. Delaying the growth of bacteria might be due to the toxic nature of AFB_1. Other species of pseudomonas, *P. aeruginosa*, was also reported to degrade AFB_1 in the nutrient medium by active cells. But only about 80% AFB_1 degradation in 72 h was achieved [33]. The present study, with culture supernatant and cell lysate, showed better degradation efficiency than the active cells, indicating that actively growing cells are not necessary for AFB_1 degradation. Similarly, Guan et al. [21] have reported better AFB_1 degradation capability of culture supernatant than the cells or cell extract of *Stenotrophomonas maltophilia*. Some reports have been conferred on aflatoxin (AF) degradation by different bacterial isolates [29, 34, 35] but a few on bacterial lysates [19, 21]. A 100% degradation of AF was observed by Teniola et al. [19] for cell-free extracts of *R. erythropolis* and *M. fluranthenivorans* in 8 h. This study thus explored the potential of *P. putida* supernatant and lysate to degrade AFB_1 over time. Culture supernatant showed good degradability of AFB_1 which can be attributed to the extracellular enzymes produced by the *P. putida*. However, more efficient degradation by the cell lysate shows the involvement of intracellular enzymes too. It was noted that lysate takes much shorter time for degradation of AFB_1 indicating that use of lysate might be a better way for degrading AFB_1. The experiment performed with heated lysate was noted to lose degrading capability, indicating protein might have been denatured on heating and hence, enzyme lost the degrading capability. The corresponding results have been previously reported by Smiley et al. [34] with *Flavobacterium aurantiacum*. In their study, the reduction in AFB_1 degradation was attributed to heat inactivation of cell-free extracts and crude protein extracts. As mentioned by Teniola et al. [19], total protein concentration can be a feasible marker for indicating the enzymatic basis for AFB_1 degradation among bacterial strains. Lysate in the presence of PMSF has more protein concentration than in absence indicating higher activity and faster AFB_1 degradation. Alberts et al. [18] demonstrated the AFB_1 was effectively degraded by extracellular extracts of *R. erythropolis* liquid culture. Results were shown from different analytical methods like LC-MS (liquid chromatography-mass spectrometry) and ES-MS (electrospray-mass spectrometry) could not reveal the formation of any breakdown products proposing that AFB_1 was most likely metabolized to degradation products with chemical properties different from that of AFB_1. Guan et al. [36] reported that AFB_1 was transformed by *Myxococcus fulvus* into a structurally different compound. Samuel et al. [29] proposed that *P. putida* strain (MTCC 2445) transformed AFB_1 into non-fluorescent compounds, AFD1 and AFD2, which are much less toxic than AFB_1 due to the opening of lactone ring of the molecule. Degradation of AFB_1 by culture supernatant and cell-free extract perhaps involves both intra- and extracellular enzymes for the reaction process. The relative protein

Table 3 Effect of temperature on AFB_1 degrading activity of *P. putida* lysate prepared in the presence of protease inhibitor (PMSF)

Temperatures (°C)	AFB_1 degradation (%) within 1 h
20	25.50 ± 0.01
30	70.50 ± 0.02
37	74.20 ± 0.03
50	82.20 ± 0.02
70	90.00 ± 0.02
100	2.80 ± 0.01

concentration of cell lysate can be used as an alternative to cell number. This study indicates the ability of culture supernatant and cell lysate in the presence and absence of protease inhibitor to effectively degrade AFB_1. Lysate showed high thermal stability as keeping at 50 °C for 24 h, it did not lose the activity. It is also stable in a wide range of pH (7–11). Lower concentrations of solvents (0.1%) and detergents (1%) also do not have a profound adverse effect on the lysate activity. In the present study, we demonstrated in vitro that lysate obtained from *P. putida* strain (MTCC 2445) was effective to remove/degrade AFB_1 in a wide range of conditions. In the future, the culture supernatant or cell-free lysate of *P. putida* can be tested for degradation of AFB_1 from contaminated food and feed matrices. However, further work is required to fully understand the mechanism and identify the enzyme responsible for AFB_1 degradation by *P. putida*, as well as related safety aspects.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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