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Evaluation of Antifungal Activity of Peptides and Enzymes Against the Sugarcane Red Rot Pathogen, *Colletotrichum falcatum*

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Abstract: In the present investigation, the antifungal activity of some peptides and enzymes were tested against *Colletotrichum falcatum*. Among them, the peptides MUC1 60mer and Purothionins mixture significantly inhibited the mycelial growth and spore germination. The peptides also showed interesting features of non-binding with DNA while displaying differential hemolytic rates on human RBCs. Comparative evaluation of MUC1 60mer and Purothionins mixture indicated that the former exhibited effective antifungal and less hemolytic activity than the latter at low concentrations. In conclusion, MUC1 60mer peptide could be a better antifungal agent with potential biotechnological applications in the treatment of sugarcane red rot infestation.

Key words: Antifungal · Sugarcane · Peptides · Red rot · Biological control

INTRODUCTION

Sugarcane (*Saccharum sp.*) is a major agricultural cash crop cultivated widely in the tropics and sub tropics. In India, it is widely cultivated in 4 million hectares with an average productivity of about 76 tonnes ha^{-1} as the main source of sugars and bioethanol [1]. Sugarcane is widely affected by biotic and abiotic factors, of which the loss to fungal pathogens is critical. Red rot caused by *Colletotrichum falcatum* Went (Perfect state: *Glomerella tucumanensis* (Speg.) von Arx and Muller) is the dreadful fungal disease which severely affects and reduces cane weight by up to 29%, loss in sugar recovery by 31%, sucrose content by 75% and juice yield by 90% [2-4]. This dual loss of juice content and quality results in great losses for both cane grower and the sugar factories. Annual loss of revenues by *C. falcatum* infection in India is estimated to be between 500 and 1000 million USD [5].

Premier management practices that tackle the red rot menace include: (a) disease resistant varieties [3]; (b) use of fungicides [6]; (c) biocontrol agents and plant-based extracts [7, 8, 37, 38]; and (d) induction of innate host resistance [9]. However, these management practices have their limitations such as high cost, side effects and decreased efficiency. Due to the complex nature of sugarcane genome, generation of resistant varieties by conventional breeding has become laborious. The fungicides, biocontrol agents and plant extracts have been effective against *C. falcatum* under laboratory conditions, but not so under the field conditions. This failure is due to the inability of the agents to reach the fungal domicile in intra cane rind [8, 10]. Also the re-emergence of new virulent strains of *C. falcatum* has resulted in the search for novel strategies for the effective control of the fungus.

Due to the rapid materialization of new pathovars, the requisite for finding new-fangled, non-toxic, compatible antifungal gene for the genetic improvement of sugarcane also increases. Antimicrobial peptides (AMP) and antimicrobial enzymes (AME) genes were earlier reported to significantly reduce the emergence of resistant pathovars [11]; increase durable disease resistance in genetically modified plants [12]; and become an alternative to fight plant pathogen infections [13]. The antimicrobial activity of peptides such as β-purothionin [14], Penicillium antifungal protein (PAF) [15],

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PGLa [16] and Hen egg white lysozyme (HEWL) [17] has already been established against certain plant pathogens. However, prior to transferring these AMP and AME genes into sugarcane genome, it is a prerequisite to establish the antagonistic activity of these molecules on the pathogen and their eventual toxicity on mammalian cells. This would be important to improve the probability of transgenic success, to reduce costs in the genetic improvement approach [18] and to establish the confidence of the stakeholders on the biosafety of the strategy [19].

The present study aims at analyzing the antifungal activity of selected AMPs and AMEs against *C. falcatum* mycelium and spores. The successful molecules that eventually showed antifungal activity were further exploited for their DNA binding and hemolytic activity.

MATERIALS AND METHODS

Chemicals: Ketoconazole, ampicillin, amphotericin B, HEWL, Potato dextrose agar (PDA) and oat meal agar were obtained from Himedia chemicals, India. Microfuge tubes (0.6ml, 1.5ml and 2ml) and 96 well flat bottom microtitre plates were obtained from Tarson Scientific limited, India. λ-DNA, RNAse A and Proteinase K were procured from Fermentas, USA. All the chemicals utilized were of analytical grade.

Peptides and Enzymes: Five peptides and three enzymes were utilized to evaluate their toxicity against the fungus and human RBCs. The list of peptides and enzymes utilized for evaluation studies are provided in Table 1.

Table 1: Peptides and enzymes investigated for antifungal evaluation against *C. falcatum*

Peptides	Origin	Source	Amino acid Sequence
Purothionins (α1, α2 and β) mixture	Wheat endosperm	Dr Elmorjani, INRA, France	α1-MGSKGLKGVMVCLLILGLVLEQVQVEGKSCCKSTLGRN CYNLCRARGAQKLCANVCRCKLTSGLSCPKDFPKLVLESNS DEPDTMEYCNLGCRSSLCDYIVNAAADDEEMKLYVEQCGDAC VNFCNADAGLTSLDA. α2-MGSKGLKGVMVCLLILGLVLEQVQVEGKSCCRT TLGRNCYNLCRSRGAQKLCSTVCRCKLTSGLSCPKGF PKLALESNSDEPDTIEYCNLGCRSSVCDYMVNAAADD EEMKLYVENCGDACVNFCNGDAGLTSLDA.
β purothionin	Wheat endosperm	Dr Ronald Skadsen, USDA, ARS, Madison	MGSKGLKGVMVCLLILGLVLEQVQVEGKSCCKSTLGRNCY NLCRARGAQKLCANVCRCKLTSGLSCPKDFPKLVLESNSDEPDT MEYCNLCRSSLCDYIVNAAADDEEMKLYVEQCGDACVNFCNA DAGLTSLDA
MUC1 60mer	Recombinant	Dr Silvia von Mensdorff-Pouilly, Academic Hospital Free University, Netherlands.	NH_2-(HGVTSAPDTRPAPGSTAPPA)3-COOH
PAF	*Penicillium chrysogenum*	Prof. Florentine marx, Innsbruck Medical University, Austria.	MQITTVALFLFAAMGGVATPIESVSNDLDARAEAGVLAKYTGK CTKSKNECKYKNDAGKDTFIKCPKFDNKKCTKDNNKCTV DTYNNAVDCD
PGLa	Frog	Dr B J Appelmelk, Vrije Universiteit, Netherlands.	GMASKAGAIAGKIAKVALKAL-NH_2
	Enzymes		Amino acid sequence
HEWL (129 AA)	Egg (*Gallus gallus*)	HiMedia, India	KVFGRCELAAAMKRHGLDNYRGYSLGNWVCAAKFESNFNT QATNRNTDGSTDYGILQINSRWWCNDGRTPGSRNLCNIPCSA LLSSDITASVNCAKKIVSDGNGMNAWVAWRNRCKGTDVQAW IRGCRL
RNAse A (128 AA)	Recombinant (Bos *taurus*)	Fermentas, USA	PSLGKETAAAKFERQHMDSSTSAASSSNYCNQMMKSRNLT KDRCKPVNTFVHESLADVQAVCSQKNVACKNGQTNCYQSYST SITDCRETGSSKYPNCAYKTTQANKHIIVACEGNPYVPVHFDASV
Proteinase K (279 AA)	Recombinant (Tritirachium album)	Fermentas, USA	AAQTNAPWGLARISSTSPGTSTYYYDESAGQGSCVYVIDTG IEASHPEFEGRAQMVKTYYYSSRDGNGHGTHCAGTVGSR TYGVAKKTQLFGVKVLDDNGSGQYSTIIAGMDFVASDKNNRN CPKGVVASLSLGGGYSSSVNSAAARLQSSGVMVAVAAGNN NADARNYSPASEPSVCTVGASDRYDRRSSFSNYGSVLDIFGPG TDILSTWIGGSTRSISGTSMATPHVAGLAAYLMTLGKTTAAS ACRYIADTA

The peptides and enzymes were dissolved in sterile MilliQ water. Penicillium antifungal protein (PAF) was dissolved in 10mM sodium phosphate buffer (pH 7.2) and stored at 4°C. Amphotericin B (50µg/ml) served as positive control and distilled water as the negative control.

Fungus Isolation: C. falcatum was isolated from the infected setts of sugarcane grown in Tiruchirappalli district of TN, India. The setts were repeatedly washed in sterile distilled water followed by surface sterilization with 70% alcohol and ketoconazole and ampicillin for 10 min respectively and finally washed with sterile distilled water to flush out the antibiotics. After surface sterilization, infected rind was split opened and scrapped. The morseled pith was plated on potato dextrose agar (PDA) (pH 5.5) and incubated at 32°C. Single spore subcultures were obtained using sterile inoculation loop, streaked on PDA medium and incubated at 32°C. After extensive sporulation, single spore was again picked by sterile fine needle and maintained on oatmeal agar with periodic sub-culturing and finally stored at 4°C.

Morphological and Cultural Identification of C. falcatum: Identification of fungus was performed according to the protocol of Abbas *et al*. [20] with slight modifications. Mycelium was scrapped from the edge of fungal colony, plated on PDA and incubated at 32°C. Diameter of the colony formation was observed consecutively for 7 days and rated as profound growth (++) and sparse (+). Sporulation was checked regularly for 7 days and recorded as mass sporulation (++) and less sporulation (+). Fungal colony colour and the production of pigments were observed. Fungal mycelium and conidia were examined under light microscope and the results were recorded.

Fungal Inoculum Preparation: Inocula were prepared as per Moreno *et al*. [21] with few modifications. The fungal mycelia were inoculated in PDA plates and incubated for 7 days. Conidial suspensions were prepared by adding sterile distilled water to the surface of the mycelium and scrapped gently. The conidial suspensions were filtered to remove the fungal mycelium and adjusted to the appropriate number using hemocytometer.

Sector Growth Pattern of C. falcatum: The sector growth pattern of *C. falcatum* was studied by inoculating the fungal spores in PDA medium and incubated at 32°C. The results were observed and recorded daily for 7 days.

Antifungal Assay: Antifungal activity of peptides and enzymes were evaluated by agar well diffusion assay. The fungal mycelia were placed at the centre of the PDA plates and incubated for 2 days at 28°C. Wells were punched at the centre of the plates in which 20 µl each of different concentrations (60µg/ml, 80µg/ml and 100µg/ml) of peptides and enzymes were loaded. The plates were incubated at 24°C for 24-48 h. The zone of inhibition around the well indicates antifungal activity of the molecules. The peptides and enzymes displaying antifungal activity were further analyzed for their hemolytic activities, sporicidal and DNA binding.

Fungicidal Kinetics and Spore Germination Efficiency: The effect of MUC1 60mer and Purothionins mixture on *C. falcatum* conidia germination was assayed as per the protocol of Kaiserar *et al*. [22]. The MIC values of MUC1 60mer and Purothionins mixture were evaluated against the germination efficiency of conidia. Briefly, 1 x 10^5 conidia were incubated with 60µg/ml of MUC1 60mer and 80µg/ml of Purothionins mixture in separate 2ml microfuge tubes for 24h at 22°C. At each time point (0, 6, 12, 18 and 24 h), aliquots were taken, diluted and plated on peptide and enzyme free PDA plates. Three replicas were maintained at each time point. The data were interpolated to derive the relationship of time versus the number of colony forming units (CFU) obtained as the function of the antifungal peptide activity.

Hemolytic Assay: Hemolytic activity of selected peptides on human RBCs was done according to the protocol of Munoz *et al*. [13]. 5ml of different blood samples (A^+, A^-, B^+, B^-, O^+, O-and AB^+) were collected from healthy persons and was subsequently centrifuged at 1,800 rpm for 3 min to pellet the RBCs and the supernatant was discarded. The final pelleted RBCs were used within 24h after bleeding and washed three times with ice cold phosphate buffer saline (PBS) (pH 7.1) to remove the hemoglobin impurities. The washing was done till the supernatant became clear.

Final pelleted RBCs were resuspended in PBS four times their volume. 90µl RBC aliquots were dispensed into 0.6ml microfuge tubes containing 10µl of varying concentrations of the active peptides, MUC1 60mer (60, 80 and 100µg/ml) and purothionins mixture (20, 40 60, 80 and 100µg/ml) respectively. The tubes were homogeneously mixed and incubated at 37°C for 1h. For each concentration and control, the experiments were set in

triplicate. A total of eight independent experiments have been performed. After the incubation, the samples were centrifuged at 1,300 rpm for 5 min and the supernatant was carefully collected, by paying attention not to disturb the pellet.

100µl samples of each supernatant were transferred to a fresh 96-well microtitre flat bottom plates. Release of hemoglobin was monitored by measuring absorbance at 415nm with a Microplate Spectrophotometer (Microquant, Biotech Instruments, USA). No-hemolysis and 100% hemolysis were determined for controls with PBS and 0.1% Triton X 100, respectively. The hemolytic activity of the peptides was calculated as percentage of total hemoglobin released to that released by incubation with 0.1% Triton X 100.

$$\% \text{ Hemolysis} = \frac{(A_{415} \text{ in the peptide solution} - A_{415} \text{ in PBS})}{(A_{415} \text{ in 0.1\% Triton X 100} - A_{415} \text{ in PBS})} \times 100$$

Electrophoretic Mobility Shift Assay: Electrophoretic mobility shift assay of peptides was performed for MUC1 60mer and Purothionins mixture to check their DNA binding capacity. Varying concentrations of MUC1 60mer (1, 3 and 6µg) and Purothionins mixture (1, 2, 4 and 8µg) were added to 1µg of λ DNA (Fermentas, USA) and incubated at 37ºC for 1 h. λ DNA without peptide treatment served as control. The peptides treated DNA and control DNA samples were then electrophoresed on agarose gels. The mobility of control DNA with that of peptide treated DNA was recorded [23].

Statistical Analysis: Each experiment was performed in three replicates. The results are presented as Mean ± Standard Error of three independent experiments.

RESULTS

Fungus Isolation and Identification: Red rot fungus was isolated from the infected stalks and identified as *C. falcatum* using cultural and morphological characters. The fungal colony showed profound growth (++) with heavy sporulation (++) and appears light orange in colour with the production of yellow pigments. Microscopic examination of fungal mycelium has revealed acervular conidiomata, thick walled and unbranched hyphae. Conidia shape found to be falcate and had fusiform apices obtuse.

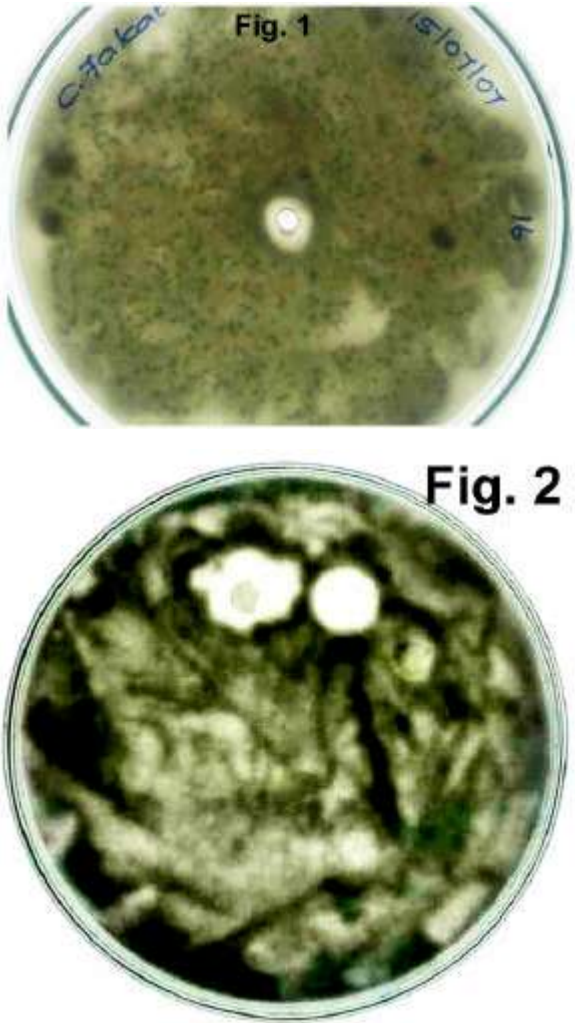

Fig. 1,2: Antifungal activity of peptides, MUC1 60mer (Fig. 1) and Purothionins mixture (Fig. 2) against *C. falcatum*.

Antifungal Assay: Agar well diffusion assay was performed on PDA plates. The zone of inhibition around the well indicated the antifungal activity of peptides and enzymes (Fig. 1 and 2). Out of the five antimicrobial peptides and three enzymes tested, two peptides such as Muc1 60mer and purothionins mixture exhibited activity against *C. falcatum* in PDA plates. The MIC of MUC1 60mer and Purothionins mixture was found to be 60µg/ml and 80µg/ml respectively. Other peptides (PGLa, β-purothionin, PAF) and enzymes (RNAse A, Proteinase K and HEWL) failed to inhibit the mycelia growth even at higher concentration. β-purothionin independently did not show any antagonistic activity. The Minimum inhibitory concentration (MIC) of peptides and enzymes was provided in Table 2.

Table 2: MIC of peptides and enzymes against *C. falcatum*

	Concentrations(µg/ml)					
Peptides and Enzymes	20	40	60	80	100	MIC(µg/ml)
MUC1-60mer	-	-	+	+	+	60
β- Purothionin	-	-	-	-	-	-
PGLa	-	-	-	-	-	--
Purothionin mixture	-	-	-	+	+	80
PAF	-	-	-	-	-	--
HEWL	-	-	-	-	-	--
RNAse A	-	-	-	-	-	--
Proteinase K	--	--	--	--	--	--

where, + indicates antifungal activity and
-indicates no activity towards *C. falcatum*.

Table 3: Determination of hemolytic activity of peptides towards human RBCs

		% Hemolysis (Mean + S.E.)					
Peptides	Concentration (µg/ml)	A^+	B^+	O^+	O^-	B^-	AB^+
MUC1-60mer	60	14.76 ± 4.3	0	9.42 ± 1.54	4.38 ± 0.25	13.3 ± 0.59	0
	80	14.52 ± 6.02	0	8.93 ± 0.56	5.15 ± 1.43	17.73 ± 2.41	1.19 ±1.26
	100	19.52 ± 4.9	0	12.8 ± 2.7	7.73 ± 0.73	21.5 ± 1.4	6.44 ± 2.09
Purothionins mixture	20	21.42 ± 4.72	22.16 ± 2.05	7.72 ± 1.25	14.94 ± 1.95	9.78 ± 1.54	22.67 ± 2.15
(α1, α2 and β)	40	23.09 ± 5.12	26.86 ± 4.09	10.14 ± 0.98	18.55 ± 0.65	12.84 ± 2.59	28.6 ± 8.1
	60	24.86 ± 4.93	34.69 ± 2.62	26.7 ± 3.17	20.06 ± 3	15.68 ± 1.94	30.5 ± 1.90
	80	40.16 ± 8.6	72.5 ± 12.9	37.1 ± 3.7	30.7 ± 5.15	36.65 ± 6.54	70.26± 16.23
	100	50.16 ± 8.60	60.87 ± 12	52.2 ± 2.55	56.13 ± 4.95	49.86 ± 1.97	79.5 ± 6.45

Fungal Sector Growth Pattern: Fan shaped sector pattern (Three and four patterns) were observed in the inoculated PDA plates (Fig. 3).

Sporicidal Kinetics: The sporicidal kinetics of MUC1 60mer and purothionins mixture on spore germination was analyzed and compared using amphotericin B as the positive control. From the graphs, it is evident that the purothionins mixture displayed equal sporicidal activity as that of the positive control till 12h, whereas the CFUs were significantly reduced at 18 and 24 h (Fig. 4). MUC1 60mer was effective in killing the spores similar to amphotericin B in 24 h. MUC1 60mer has shown high sporicidal activity at 12h and extend up to 18h than amphotericin B (Fig. 5). From the graphs (Fig. 4 and 5), it has been evident that MUC1 60mer has shown high sporicidal activity than purothionins mixture.

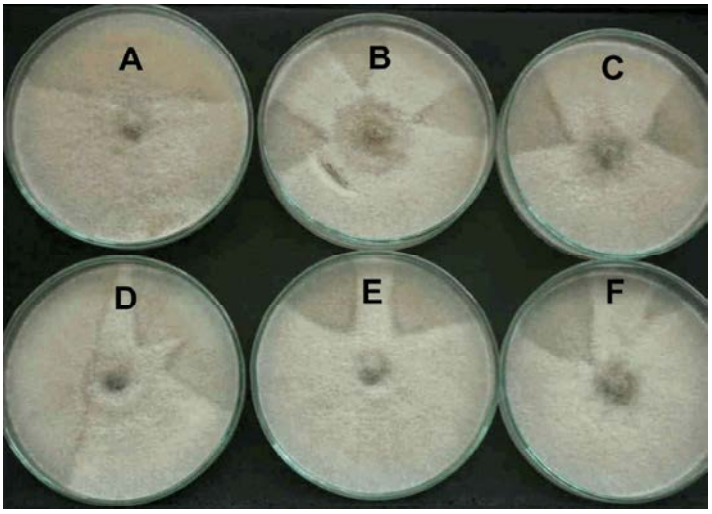

Fig. 3: Sector growth pattern of *C. falcatum* on PDA medium

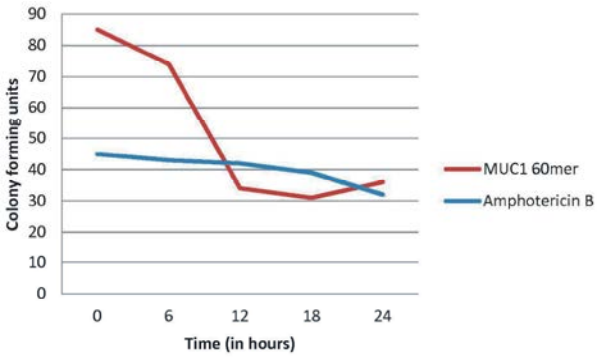

Fig. 4: Sporicidal activity of MUC1 60mer peptides

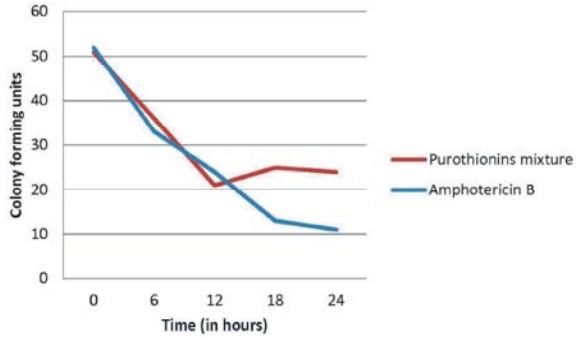

Fig. 5: Sporicidal activity of Purothionins mixture

Hemolytic Assay: The effect of peptides on human RBCs was investigated at different concentrations (20, 40, 60, 80 and 100 µg/ml). The hemolytic rate of MUC1 60mer and purothionins mixture on different human RBCs was compared to determine the mammalian cell toxicity, if any. The results have been presented in Table 3. It was observed that increase in concentration of peptides had differential hemolytic activity in the blood groups. MUC1 60mer exhibited less hemolysis than purothionins mixture. MUC1 60mer displayed less hemolytic activity (<10%) towards B^+, O^+, O-and AB^+ RBCs whereas A^+ and B-RBCs showed >10% hemolytic activity. MUC1 60mer showed 14-20% lysis of human A^+ and B-erythrocytes. However, less hemolysis (<10%) was observed in other RBCs (B^+, O^+, O-and AB^+). MUC1 60mer exerted maximum activity on all blood cells at 100µg/ml concentration except on B^+, which showed no hemolysis. Increasing concentrations of MUC1 60mer resulted in less hemolytic activity in B^+ and B-RBCs. The hemolysis by MUC1 60mer was less (2-13%) compared to purothionins mixture on all blood groups.

Purothionins mixture showed hemolysis (7-80%) for all RBCs. At 100 µg/ml concentration, 80% hemolysis of AB^+ RBCs was observed whereas 50-60% hemolysis was observed in other RBCs (A^+, B^-, B^+, O^+, O^-). Purothionins showed similar hemolytic activity on A^+ and B^+ RBCs and O^+ and O-RBCs. Increase in the concentration of purothionins mixture greatly increased the hemolytic activity (20-80%) of the RBCs. Comparatively purothionins treated B^+ and AB^+ RBCs showed corresponding hemolytic rate in all the concentrations utilized. At 100µg/ml, A^+, B^-, O^+, O-showed equivalent hemolytic rate.

Electrophoretic Mobility Shift Assay: The MUC1 60mer and purothionins mixture were analyzed for their ability to bind non-specifically to λ-DNA. No binding of peptides to λ-DNA was observed in agarose gel electrophoresis. The peptide treated DNA samples compared to untreated DNA showed no differences in the migration and separation of DNA in agarose gel electrophoresis.

DISCUSSION

Red rot disease inflicts severe economic losses for both sugarcane farmers and sugarcane industries. Hence strategies have to be designed to control the fungal infections. In the present investigation, red rot fungus was isolated from infected setts, identified and confirmed as *C. falcatum* by morphological and cultural based methods. Our results were consistent with the morphological and cultural identification of *C. falcatum* as reported earlier [24-26].

Compared to other antifungal evaluation methods, agar well diffusion was effective since the peptides were in contact with the developing mycelia around the well and the growth inhibition can be easily observed. MUC1 60mer and Purothionins mixture has shown antagonistic activity against *C. falcatum* in agar well diffusion. Purothionins mixture consists of α1, α2 and β purothionins [27]. No zone of inhibition was observed in β purothionins loaded wells. However there was a clear zone of inhibition observed in purothionins mixture loaded wells. This indicates that β purothionins doesn't have any antifungal activity, but the antifungal activity of the purothionins mixture may be due to the activity of either α1or α2 or synergistic action of both α1 and α2 purothionins. This is consistent with the published report of Mackintosh *et al*. [28] stated that α1 purothionin transgene in transgenic wheat significantly reduced *Fusarium* head blight disease. In our investigation, the antifungal activity displayed by MUC1 60mer and purothionins mixture peptides was lower than that of amphotericin B. These peptides are believed to kill the fungus either by disrupting the plasma membrane or by

interacting with the DNA by penetrating the nucleus as implicated in yeast apoptosis [29]. However, the exact killing mode of peptides on *C. falcatum* is yet to be explored.

The sector growth pattern of *C. falcatum* could be an adaptive strategy against temperature variations or nutrition content of the medium. This is consistent with the results of Malathi *et al*. [30] proposed the similar growth patterns in *C. falcatum* when repeatedly inoculated on incompatible sugarcane cultivars, re-isolated and cultured on agar medium.

MUC1 60mer significantly killed the spores thus affecting its germination and colony forming units than Purothionins mixture. Purothionins mixture inhibited the growth of the fungal spores effectively in a manner similar to that of amphotericin B. Over the course of time, significant reduction of CFU was observed in peptide treated spores than amphotericin B treated. This is consistent with the report of Carlson *et al*. [31] stated the inhibition of fungal spores by barley hordothionin. The sporicidal activity of these two peptides could be due to its binding with fungal membrane glucosylceramides, inhibition of sphingolipid mannosyl di-inositolphosphoryl ceramide biosynthesis, swell and burst the hyphal tips leading to the leakage of intracellular constituents and inhibit the spore germination [32, 33].

The hemolytic activity of peptides was assayed to ensure their safety towards humans/ animal cells. The two peptides that showed better antifungal activity were further analyzed for their hemolytic activity. Variation in hemolysis rate was observed among the MUC1 60mer and purothionins mixture treated blood RBCs. It may be concluded that MUC1 60mer has less toxicity for mammalian cells compared to purothionins mixture, as reported earlier by Teeter *et al*. [34]. The hemolytic activity of purothionins may be due to the interaction of positively charged purothionins with negatively charged phospholipids present in the cell membranes, thus leading to pore formation and leakage of cytoplasmic contents [35].

AMPs are mostly positively charged and have the capacity to bind negatively charged DNA molecules [36]. As the peptide binds to the DNA, the molecular weight of the complex increases and there will be a shift in the electrophoretic mobility of the DNA. No difference was observed in the electrophoretic mobility of peptide treated DNA compared to the control. This showed that the two peptides cannot bind to the DNA and they disrupt only the plasma membrane of the growing fungus thereby bringing about fungal death.

Based on the above results, it has been speculated that the MUC1 60mer gene could be compatible for the genetic improvement of sugarcane to impart disease resistance against red rot fungus.

CONCLUSION

Red rot disease caused by *C. falcatum* is one of the major devastating diseases of sugarcane. Compared to other methods, genetic improvement of sugarcane with foreign genes could offer the vital solution to sustain durable resistance against red rot disease. AMPs are attractive agents for the genome enhancement for many reasons. They are lithe to pH changes; target the pathogen effectively with minimal or no toxicity to host and mammalian cells; prevent the emergence of new pathovars. In this investigation, *C. falcatum* mycelium and spore germination was found to be inhibited greatly by MUC1 60mer than purothionins mixture. MUC1 60mer showed less hemolytic activity than purothionin mixtures thus revealing its non-toxicity against mammalian cells. Both peptides have shown no affinity towards DNA. The effect of MUC1 60mer on mycelial inhibition, spore killing, lower hemolytic activity and non binding to DNA, suggests this AMPs could be an ideal gene for the genetic improvement of sugarcane in controlling red rot disease. Future perspective includes the genetic transformation of the MUC1 60mer peptide genes into sugarcane genome to impart disease resistance.

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