ISSN : 0974 - 7435 Volume 11 Issue 1

BioTechnology
An Indian Journal

FULL PAPER

BTAIJ, 11(1), 2015 [26-28]

Expression of virulence-related *abr*1 gene of *Aspergillus fumigatus* treated with *Acorus calamus* extract and asarones

S.Ashadevi, Anamika Das, Shivani, Shiji Mathew Zacharia, Subramanian Babu*
School of Bio Sciences and Technology, VIT University, Vellore 632014, (INDIA)
E-mail : babu.s@vit.ac.in

ABSTRACT

Effect of the rhizome extract and α- and β- asarones (the chemical analogues of the major bioactive constituents of the extract) on expression of *abr*1, a regulatory gene in *A. fumigatus* melanin biosynthetic pathway is reported in the present study. Reverse transcription PCR analysis revealed inhibition of gene expression by α- and β- asarones compared to crude rhizome extract and the standard antifungal drug.

KEYWORDS

Abr1 gene;
Acorus calamus;
Asarone;
Aspergillus fumigatus;
Rhizome.

INTRODUCTION

Resistance of human pathogenic *Aspergillus* to available drugs is on the rise with significant potential implications for the management of invasive aspergillosis[1]. While research on developing new antifungal weapons like posaconazole is encouraging[2], search for antifungals in medicinal plants is also on in an extensive way, as an alternative to chemical drugs. *Acorus calamus* L. is a well known plant in Indian traditional medicine and reported to possess antifungal activities[3]. Both α-and β-asarone are indentified as the major chemical constituents in roots, rhizomes, leaves and essential oil of this plant responsible for all the biological activities. Previously, we have reported the antifungal activity of *A. calamus* rhizome as well as the purified α-and β-asarones on human pathogenic *A. fumigatus*[4]. Here we report the effect of rhizome extract of *A. calamus* as well as the α -and β-asarones on the expression of *abr*1, an important gene in the melanin biosynthetic pathway.

EXPERIMENTAL

A. calamus plants were collected from Horticultural Research Station, Yercaud, Tamil Nadu, India and grown in the herbal garden of VIT University, Vellore, India. Rhizome extract was prepared as described previously[5].

Stock solutions of *A. calamus* rhizome extract, commercial α- and β- asarone (Sigma, St Lois, USA) and itraconazole (antifungal drug) were prepared in DMSO and added to the *A. fumigatus* minimal media[6] to achieve a final concentration of 200 µg of rhizome extract, 100 µg of α-asarone, 100 and 200 µg of β-asarone and 1 µg of itraconazole per ml. Culture discs from actively growing *A. fumgatus* culture (MTCC343, IMTECH, Chandigarh, India) were used for inoculation. Cultures in minimal media with DMSO and without any treatment served as control. Visual observa-

tions on growth, pigmentation and mycelia biomass were made. Total RNA was isolated using RaFlex Kit (Genei, Banglore, India) and cDNA was prepared using oligo d(T) primer (cDNA synthesis kit, Genei, Bangalore, India) as per the manufacturer's instructions. PCR was carried out in thermal cycler (Eppendorf, Germany) with 35 cycles of denaturation at 95p C for 1 min, annealing at 46p C for 1 min and extension at 72p C for 1 min. The primer sequences are: 5'GCGTCGATATACGGTCAGGT3' and 5'GGGACAGATTCGCTGATGAT3'.

RESULTS AND DISCUSSION

The results of visual observation and the mycelial biomass of *A. fumigatus* grown under different treatments are given in TABLE 1. The α-asarone treatment suppressed the growth of mycelia and no visible fungal mat was found. The β-asarone treatment resulted in bleaching effect leading to yellowish green mat compared to the dark green in untreated control. Treatment with antifungal drug was similar to control and there was no visible change in the color. However, *A. calamus* rhizome extract treatment exhibited pale green color mycelia. The fungal biomass produced was significantly reduced in α-asarone treatment. RT-PCR results are shown in Figure 1. Expected size amplification of approximately 400 bp was observed in untreated control, rhizome extract and itraconazole treatment. Based on presence and absence as well as intensity of the amplified fragment observed in gel for the normalized 200 ng RNA, both α- and β-asarone were found to be inhibiting the *abr*1 gene expression.

Some reviews have focused on the synthesis of melanin in pathogenic fungus and its importance[7-9]. In the present study, the asarone which are the chemical analogs of the major components present in *A. calamus*, inhibited the expression of *abr*1, the key regulatory gene in melanin biosynthesis. However, the crude extract of *A. calamus* rhizome which consists of other

TABLE 1: Visual observations of growth and mycelia biomass of *Aspergillus fumigatus* under different treatments

Treatments	Growth observed	Mycelial wet weight (g)*
Control (untreated)	Dark green colour, thick mat observed on the surface of the media	4.64
α-asarone (10 mg)	No mat observed.	0.61
β-asarone (10 mg)	Light yellowish green mat observed in the media	3.23
β-asarone (20 mg)	Light yellowish green mat observed in the media	3.03
A. calamus rhizome extract (10 mg)	Dark green colour, thick mat observed on the surface of the media	4.30
Itraconazole (10 mg)	Green colour mat observed on the surface of the media	3.91

*** Mean of three replications**

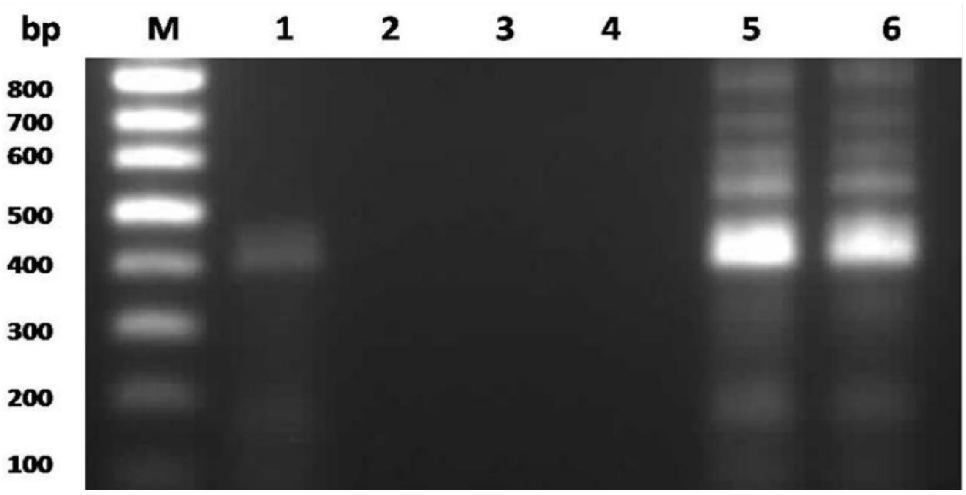

Figure 1 : RT-PCR analysis of *Aspergillus fumigatus abr*1 gene expression; Lanes M – molecular weight marker; 1 – untreated control; 2 – 10 mg α-asarone; 3 – 10 mg β-asarone; 4 – 20 mg β-asarone, 5 - *A. calamus* rhizome extract; 6 – itraconazole

compounds in addition to asarones did not exhibit suppression of expression. The antifungal drug also had no effect on the expression of the gene. The result reveals that the purified form of asarones from *A. calamus* may have the suppression effect on melanin biosynthesis in fungi and thus on the virulence. The other compounds that may be present in the rhizome extract are found to be inhibitory to the antifungal activity of asarones in the extract. Hence, these asarones can further be characterized, purified in large quantities and used to develop antifungal drug against *A. fumigatus.*

ACKNOWLEDGEMENT

The support by the management of VIT University, Vellore, India, to carry out the research work is also gratefully acknowledged.

REFERENCES

[1] S.P.Georgiadou, D.P.Kontoyiannis; Ann.N.Y.Acad. Sci., **1272**, 15-22 (**2012**).

[2] G.Aperis, P.Alivanis; Rev.Recent Clin.Trials, **6**, 204-219 **(2011)**.

[3] J.Y.Lee, J.Y.Lee, B.S.Yun, B.K.Hwang; J.Agric.Food Chem., **52**, 776-780 **(2004)**.

[4] S.Ashadevi, D.Ganjewala; Acta Biol.Szeged., **53**, 45-49 **(2009)**.

[5] S.Mehrotra, K.P.Mishra, R.Maurya, R.C.Srimal, V.S.Yadav, R.Pandey, V.K.Singh; International Immunopharmacol., **3**, 53-61 **(2003)**.

[6] M.L.Medina, P.A.Haynes, L.Breci, W.A.Francisco; Proteomics, **5**, 3153-3161 **(2005)**.

[7] K.Langfelder, M.Streibel, B.Jahn, G.Haase, A.A.Brakhage; Fungal Genet.Biol., **8**, 43-158 **(2003)**.

[8] S.E.Baker; In *Aspergillus in the genomic era*. Wageningen Academic Publishers, The Netherlands, 73-85 **(2008)**.

[9] M.P.Pihet, P.Vandeputte, G.Tronchin, G.Renier, P.Saulnier, S.Georgeault, R.Mallet, D.Chabasse, F.Symoens, J.P.Bouchara; BMC Microbiol., **9**, 177 **(2009)**.