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SHORT COMMUNICATIONS

Evaluation of DNA Methylation Changes by CRED–RA Analysis Following Prednisone Treatment of Endophyte, *Fusarium oxysporum*

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Abstract Endophytes that represent a sub-set of plant resident microbes are a reservoir of bioactive metabolites. Many of the secondary metabolite biosynthetic gene clusters of endophytes are silent under axenic culture conditions. Epigenetic reprogramming of such cryptic pathways is possible by use of small molecule modulators like prednisone. Methylation changes induced by prednisone, a hypomethylating epigenetic modulator were studied in endophytic *Fusarium oxysporum*. CRED–RA analysis following exposure to non-cytotoxic dose (300 µM) revealed prednisone as effective in inducing non-methylation and semi-methylation pattern while inhibiting full-methylation of the genome. Effectiveness of prednisone as a DNA methyl transferase inhibitor can be explored in future to study alterations in secondary metabolite gene expression profile in endophytic *F. oxysporum*.

Keywords *Fusarium* · Prednisone · CRED–RA · RAPD

Introduction

Biosynthetic potential of endophytes from medicinal plants represents an attractive source for discovery of new bioactive metabolites. *Zingiber zerumbet* commonly named pinecone or shampoo ginger is one such medicinal plant of research interest due its wide range of pharmaceutical activities [1]. Our earlier studies had characterized *Z. zerumbet* endophytic repertoire and identified the endophyte designated *ZzEF*8 as exhibiting inhibitory activity against *Pythium myriotylum*, an economically significant phytopathogen [2]. Metabolite analysis of *ZzEF*8 identified as *F. oxysporum* by ITS/16S rRNA analysis [2] detected umbelliferone as major metabolite responsible for anti-*Pythium* and cytotoxic effects (unpublished). Umbelliferones synthesized via phenylpropanoid pathway are phytoalexins produced by plants following infection with pathogenic fungi [3, 4]. While umbelliferone production by numerous plant species have been reported earlier, reports on production of umbelliferone from endophytes are scarce [5, 6].

Comparative genome analyses indicate *Fusarium* species to have significant genetic potential to produce wide range of secondary metabolites. Secondary metabolite biosynthetic genes in filamentous fungi are organized as gene clusters with many being either silent or expressed at very low levels under axenic culture conditions [7–9]. Earlier studies had used epigenetic modulators for pleiotropic activation of cryptic or silent secondary metabolite biosynthetic genes [10] resulting in the production and/or enhancement of bioactive compounds that would otherwise not be produced under normal growth conditions [11]. Use of prednisone, a small molecule epigenetic modulator for regulation of secondary metabolite gene cluster(s) in endophytes has been studied in *Pestalotiopsis fici* for production of polyketides like pestaloficiols T–W and macrodiolide ficiolides A–K [12] and *Bacillus pumilus* strain RI06-95 for lipopeptide antibiotics [13]. Prednisone, a corticosteroid, is a DNA hypomethylating agent regulating epigenetic modifications by inhibiting DNA methyltransferase (DMT) or histone methyltransferase (HMT) [14]. DNA methylation that adds methyl group to cytosine in CpG islands can be detected by various

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