Endophytic Xylariaceae from Thai Plants: A Research Review

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Abstract: Endophytic fungi are present in all plant species investigated, and members of the Xylariaceae are especially common in tropical plants. There is major interest in endophytic fungi, because of their excellent track record concerning the production of novel and often bioactive compounds including the anti-cancer drugs taxol and taxane. Studies in Thailand on endophytes over the past 20 years confirm the high presence of the Xylariaceae in many different Thai plants. Species of *Xylaria* stand out as the most frequent Xylariaceae isolated; although, *Daldinia eschscholtzii* has also been commonly isolated. DNA technology and chemical profiling have been shown to be invaluable in the identification of xylariaceous isolates which previously could not be identified to species level or even assigned to genera in many cases. Reference is made to those isolates which produce novel compounds or those exhibiting bioactive properties.

Keywords: endophytic fungi, xylariaceae, Thailand, novel metabolites.

Introduction

Endophytic fungi have been described as those organisms that live inside the plant tissue for at least part of their life cycle without causing any disease symptoms in the host (Petrini, 1991). There is currently major interest in endophytes mainly following the discovery of the anticancer drugs taxol and taxane in the endophyte *Taxomyces andreanae* Strobel, A. Stierle, D. Stierle & W. M. Hess (Stierle *et al*., 1993) and subsequent references to bioactive compounds isolated from endophytes (Strobel *et al*., 2004; Tenguria *et al*., 2011). It is noteworthy that the Xylariaceae are well known as a source of novel metabolites (Whalley & Edwards, 1995; Stadler & Hellwig, 2005) but also exhibit a remarkable presence as endophytes being regular and often dominant members of the endophytic communities of tropical plants (Petrini *et al*., 1995). It is therefore not surprising that the Xylariaceae have been the target for many researchers. In Thailand, there have been a number of important studies with numerous publications on both novelty of the chemicals produced and their bioactive properties. Early studies on xylariaceous endophytes encountered difficulties in identification, certainly to species level, since they fail to produce stable diagnostic features and most of the anamorphic endophytic isolates failed to develop their teleomorph in culture. In most cases, the cultures and anamorphs are insufficiently distinctive to enable confident identification but pioneering work by Petrini and colleagues (*e.g*. Petrini, 2013; Petrini & Petrini, 1985; Petrini *et al*., 1995) has resulted in the development of keys and publication of suitable data to allow identification to be made at least to generic level for temperate isolates. The situation regarding tropical endophytic Xylariaceae is much more complex as a result of their abundance, almost universal presence and their impressive diversity (Rodrigues & Samuels, 1990; Rodrigues, 1994; Whalley, 1996). It is doubtful whether differentiation of species on the basis of cultural and anamorphic features alone will ever be possible since differences between individual species are often insufficient to allow

for absolute identifications to be made (Petrini *et al*., 1995). Attempts were made to use a combination of morphological and biochemical data and these were considered to be promising (Brunner & Petrini, 1992; Rodrigues *et al*, 1993). During this period, investigations into secondary metabolite profiles from endophytic isolates might be 'matched' with those obtained from cultures derived from teleomorphic material thus enabling identity to be established (Whalley & Edwards, 1995; Whalley, 1996). In a few cases, identification could be made on the basis of metabolite profiles compared with those from known teleomorph forms. Since then, Stadler and his many co-workers have developed a sophisticated database of secondary metabolites occurring in an impressive range of xylariaceous taxa using HPLC-DAD/MS profiling, HPLC-DAD/MS de-replication, GC-MS profiling and NMR spectroscopy (Stadler *et al*., 2001a, 2001b, 2008, 2010). A recent monograph of the genus *Daldinia* Ces. & De Not. combining traditional taxonomic characteristics with chemical and molecular data provides powerful evidence for this approach and the great future potential for the identification of these previously unidentifiable xylariaceous endophytes (Stadler *et al*., 2014).

In this mini review, we refer to important aspects of the lifestyle of endophytic fungi concentrating on our studies in Thailand over the past 20 years. Previously unpublished data is included and is discussed in the light of other studies on endophytic fungi.

Endophytic Xylariaceae of Thailand

In early studies of endophytes of *Tectona grandis* L (teak), attempts were made to produce the teleomorphs of a range of endophytic Xylariaceae to enable identifications to be made. It was found that inoculation of pre-sterilized twigs with pure cultures isolated from mature stromata on decaying plant material collected from the sites where endophytic isolates had been obtained or from cultures of selected xylariaceous endophytes which when placed in the forest to develop could often produce mature stromata (Mekkamol *et al*., 1997; Mekkamol, 1998). *Daldinia eschscholtzii* (Ehrenb.) Rehm took 12-14 weeks. but species of *Xylaria* took over 24 weeks or longer to maturity. *Xylaria grammica* (Mont.) Mont., *X. cubensis* (Mont.) Fr. and *D. eschscholtzii* were subsequently recognized from mature stromata developing in twigs inoculated with endophytic isolates. Interestingly *D. eschscholtzii* was found to be the dominant endophyte in teak leaves sampled early in the rainy season with *Xylaria* later becoming more frequent (Mekkamol *et al*., 1997; Mekkamol, 1998). It is suggested that this was a result of the higher inoculum potential of the *Daldinia* ascospores following the development of mature stromata early in the rainy season and its ability to produce large numbers of spores. *Xylaria* species were found to take longer to develop to maturity and this might explain their low presence early in the rainy season but their greater frequency later on. Our more recent studies confirm that *D. eschscholtzii* is a frequent endophyte in Thai plants Chareprasert *et al*., 2006, 2010, 2012). In their studies on endophytes of wild banana (*Musa acuminata*) at Doi Suthep Pui National Park (Photita *et al*., 2001) and from *Amomum siamense* Xylariaceae were also common endophytic fungi (Bussaban *et al*., 2001). A review of endophytic fungi in Thailand gave insight into their diversity and potential for future investigations (Lumyong *et al*., 2004) Endophytes from *Garcinia* species also exhibited high xylariaceous representation (Phongpaichit *et al*., 2006). A major study of endophytic Xylariaceae in Thailand was then undertaken by Okane *et al*. (2008) in which 405 strains of Xylariaceae (273 endophytic and 132 saprobic strains) were studied to examine the diversity and taxonomy of endophytes and to explore the relationships between those endophytes and saprobic Xylariaceae in Thailand recognized by their teleomorphic characteristics. In this study in KhaoYai National Park, it was found that *Xylaria* species were dominant and in analysis of 28S rDNA D1/D2 sequences 21 species of Xylariaceae were recorded inhabiting tropical plant foliage. Apart from *Xylaria, Hypoxylon haematostroma* Mont. and *D. eschscholtzii* were confirmed endophytic isolates. Our own studies from various sites and host plants in Thailand support these findings with *Xylaria* being by far the dominant xylariaceaous genus in most cases (Mekkamol *et al*., 1997; Chareprasert *et al*., 2006, 2010, 2012; Pharamat *et al*., 2013;Ruchikachorn, 2005).

Whalley (1996) reported *Anthostomella* Sacc., *Biscogniauxia* Kuntze, *Daldinia* Ces., *Hypoxylon* Bull., *Kretzschmaria* Fr., *Nemania* Gray, *Rosellinia* De Not. and *Xylaria* Hill ex Schrankto have endophytic representation. Members of these genera have been confirmed as endophytes in Thai plants and selected representatives are shown in Figure 1. Other genera since recorded

include *Annulohypoxylon* Y.-M. Ju, J.D. Rogers & H. M. Hsieh and *Muscodor* Worapong, Strobel & W. H. Hess. The genus *Muscodor albus* anam. gen. et sp nov. was erected for an endophytic isolate from *Cinnamomum zeylandicum* and is included in the Xylariaceae on the basis of its molecular similarities (Worapong *et al*., 2001). It appears to be widely distributed in tropical rainforests and has been recorded from Thailand (Sopalun *et al*., 2003). The following new species of *Muscodor* have since been described from Chiang Mai Province *M. cinnamomi* Suwannarach, Bussaban, K. D. Hyde & Lumyong, *M. musae* Suwannarach & Lumyong, *M. oryzae* Suwannarach & Lumyong, *M. suthepensis* Suwannarach & Lumyong and *M. equiseti* Suwannarach & Lumyong (Suwannarach *et al*., 2010; 2013). *Muscodor* species are of considerable interest since they produce volatile metabolites with promise for biocontrol of spoilage fungi (Strobel, 2010).

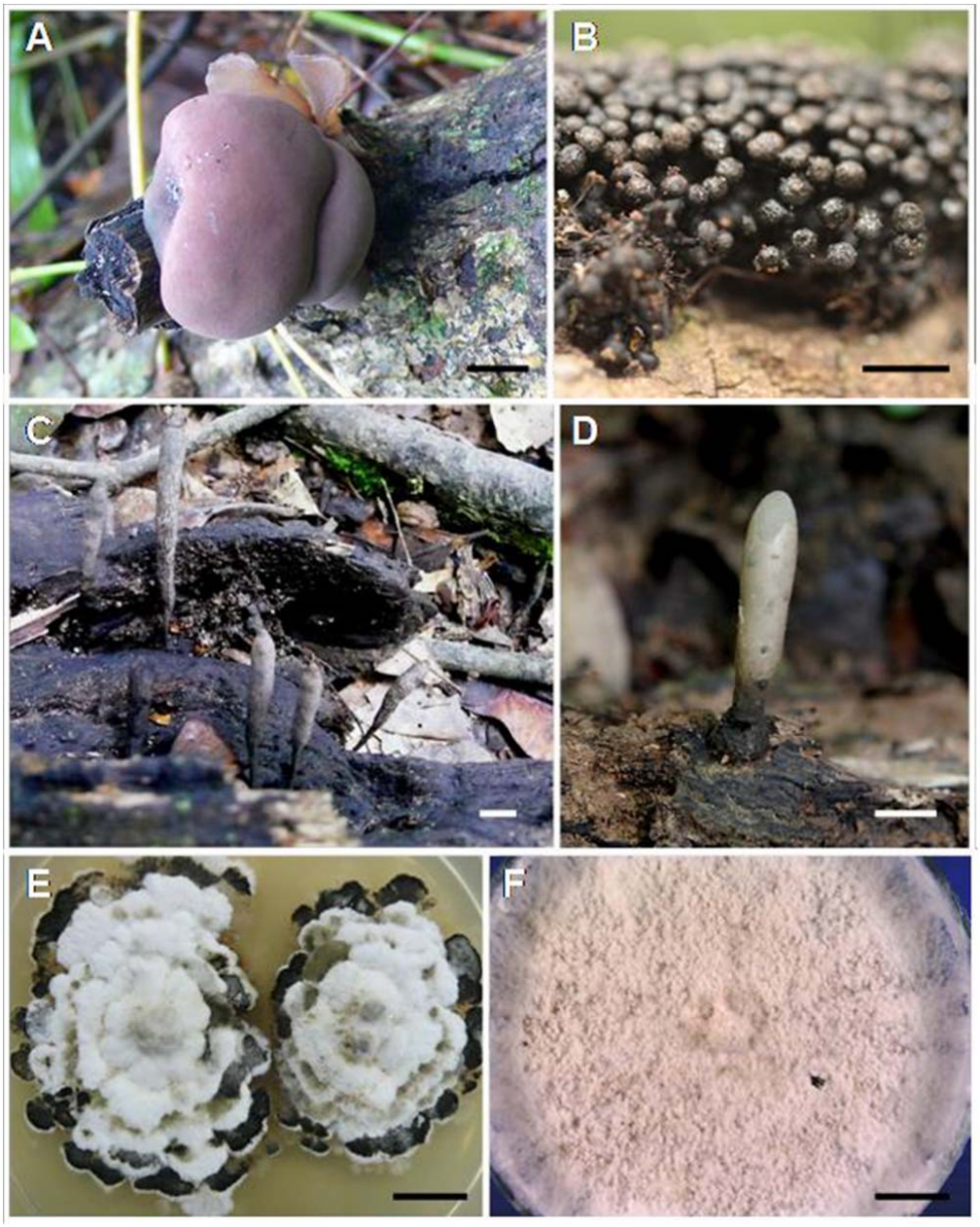

Figure 1. Selected xylariaceous fungi. A. *Daldinia eschscholtzii*, B. *Kretzschmaria* sp., C. *Xylaria grammica*, D. *Xylaria cubensis*, E. *X. grammica* culture on PDA medium, F.*X. cubensis* culture on PDA medium. Bars = 1 cm.

Novel chemicals and bioactivity

Our early studies on secondary metabolites of the Xylariaceae demonstrated a remarkable diversity of chemicals with many proving to be novel structures (Adeboya *et al*., 1995a, 1995b; Anderson *et al*., 1982, 1983, 1984a, 1984b, 1985; Edwards & Whalley, 1979; Edwards *et al*., 1988, 1989, 1991, 2001). It was also shown that the presence or absence of certain metabolites and metabolite profiles were of value in making taxonomic judgement (Whalley & Edwards, 1995). Since this early work, Stadler and co-workers have identified numerous new compounds and in many cases have demonstrated useful bioactive properties (Stadler *et al*., 2001a, 2001b, 2008, 2010; Bitzer *et al*., 2008). Their results have proven to be invaluable in both species identification and in taxonomic classification. These publications, together with the discovery of taxol and taxane in an endophyte, have resulted in wide ranging investigations of meta-bolites from the Xylariaceae and xylariaceous endophytes. There are too many publications to list all here, but a few selected papers on bio-active properties by Daferner *et al*., 1999; Singh *et al*., 1999; Isaka *et al*., 2000; Quang *et al*., 2006 provide a useful base to work from. Sodngam *et al*. (2014) investigated the chemicals and their activity from *Xylaria humosa* Lloyd recently found in Thailand and provide key references to bioactive compounds from *Xylaria* species. Stadler and Hellwig (2005) have reported on a wide range of chemicals identified and their bioactive properties.

Conclusions

The Xylariaceae are common and widely distributed endophytes in those Thai plants which have been investigated. The genus *Xylaria* is dominant in most of the studies undertaken but *D. eschscholtzii* is also a regular inhabitant and in certain situations can be numerically the most important. There are a large number of isolates recognized as xylariaceous without identification to genus and species levels. *Xylaria* endophytes are in particular a good source of metabolites, many novel and a number exhibit bioactive properties.

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