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Endophytic fungi harbored in Chinese native gramineous plants

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Abstract: Epichloë endophytes, including *Neotyphodium* spp. and *Epichloë* spp., enhance plant growth, mediate more plant tolerance or resistance to biotic and abiotic stresses, and also synthesis various biologically active compounds in their host plants, and important in many areas. In early stages, most of epichloë endophytes were described during surveys practiced by American, European and Oceania scientists, while fungal endophytes within native Asian plants were poorly investigated. In recent years, an *Epichloë* sp. and 4 *Neotyphodium* spp. were described in cool season Chinese native gramineous plants. Most of Chinese native *Neotyphodium* spp. were presumed as hybrids originated from members of ETC and EBY. Investigation on NRPS genes shows lack of toxic ergopeptines and potential production of peramine. Biological and ecological roles of Chinese native epichloë endophytes should be investigated in future, and it will be very valuable if we can have some joint projects with Korean scientists for Asian native epichloë endophytes.

Key words: epichloë endophytes; *Neotyphodium* spp.; *Epichloë* spp.; Phylogenetic analyses; NRPS genes

Some epichloë endophytes, including *Neotyphodium* spp. and *Epichloë* spp., enhance plant growth, mediate more plant tolerance or resistance to biotic and abiotic stresses, and also synthesis various biologically active compounds in their host plants (White et al., 1993; Schardl et al., 2004; Kuldau and Bacon. 2008). Such grass-endophyte symbiosis is important in many areas, especially in plant breeding for turf and conservation purposes (Clay 1989; Malinowski & Belesky, 2000). *Epichloë* spp. occasionally produce stromata on the culms of host plants and prevent heading of the inflorescence, so they are recorded as pathogenic fungi in plant

pathology. *Neotyphodium* spp., known as "asymptomatic" endophytes, do not produce any symptom on their host plants in most cases. Both *Epichloë* spp. and *Neotyphodium* spp. spend their most of life cycles within their hosts and do not kill or penetrate living host plant cells, so both of them are "endophytic" (White et al., 1993; Schardl et al., 2004; Shen et al., 2009). Recently, 22 species and 5 variations in genus *Neotyphodium* and 11 species in genus *Epichloë* had been described respectively all over the world (Schardl et al., 2004; Li et al., 2004; Li et al., 2006; Moon et al., 2007; Ji et al., 2009; Kang et al., 2009; Chen et al., 2009).

Last century, most of epichloë endophytes were described during surveys practiced by American, European and Oceania scientists, while fungal endophytes within native Asian plants were poorly investigated (Table 1). By using of plant materials collected in China, Wilkinson *et al.* firstly found endophytic fungi in 1991 from a *Hordeum* germplasm collections (Wilkinson et al., 1991), Miles *et al.* detected an *Acremonium*–like endophytic fungus from *Achnatherum inebrians* (Hance) Keng, the so-called "drunken horse grass" in China, accompanied by high levels of toxic alkaloids (Miles et al., 1996). B. Li *et al.* detected 3 grass species harboring endophytic fungus out of 10 stored in the Xinjiang Academy of Animal Sciences (Li et al., 1997). In Japan, Koga et al. studied epichloë endophytes in cultivated forage grasses including *Lolium* spp., and *Festuca* spp. (Koga et al., 1993; 1995). From Japanese native grasses, Z. Wang *et al.* reported existence of an *Acremonium* (=*Neotyphodium*) endophyte in *Agropyron* (*Roegneria*) *kamoji* in 1997 (Wang et al., 1007), Mr. Yu, a PhD. candidate in University of Tokyo came from Korea, reported an *Acremonium* endophyte from *Poa pratensis* L. with his colleagues (Mr. Yu, personal communication, 1997), and Imada and Shinozaki *et al.* demonstrated a *Acremonium* (=*Neotyphodium*) endophyte from plants of *P. trivialis* L. (US Patent). Later, Yanagita and Tajimi *et al.* reported existence of an *Epichloë* endophyte from plants of *Brachypodium sylvaticum* (Huds.) Beauv. (Yanagida et al., 2005). In China, an *Epichloë* species and several *Neotyphodium* species were erected from cool-season grasses. The first one was the *Neotyphodium gansuense* Li et Nan, harbored in *A. inebrians* grown in Northwest of China, was first erected based on their morphological properties in 2004(Li et al., 2004). *Epichloë yangzii* Li et Wang, a stroma-producing endophyte from *Roegneria* plants grown in east basin of Yangtze River, reported in 2006 as the first sexual endophyte. It was interfertile with *E. bromicola* Leuchtmann et Schardl, but genetic phylogenetic evidences, geographic distribution and host specificity supported *E. yangzii* to be a new species (Li et al., 2006a; Li et al., 2006b). This species is now an important member of EBY, a phylogenetic clade consisted of *E. bromicola* and *E. yangzii* (Li et al., 2006b). The third one was the *Neotyphodium sinicum* Wang, Ji et Kang, an asymptomatic endophyte from *Roegneria* plants grown in a wide area in China including in Jilin, Xinjiang and Fujian, reported

in 2009 (Kang et al., 2008a; 2009). Phylogenetic evidences supported that this species should be an interspecific hybrid of ETC (*E. typhina* clade) and *E. yangzii*, the only member of EBY distributed in China (Kang et al., 2008b; 2009). The forth one was *N. stromatolongum* Ji, Zhan et Wang, a stromata producing *Neotyphodium* species, because all of stromata resulted by species on culms of host plants were infertile (Zhan et al., 2008; Ji et al., 2009). It was harbored in infertile *Calamagrostis epigeios* (L.) Roth. plants grown in Nanjing (Zhan et al., 2008). This is a non-hybrid *Neotyphodium* species and transmitted by the clonal propagation of the host (Ji et al., 2009). The fifth one was the *N. sinofestucae* Chen, Ji et Wang, another asymptomatic hybrid endophyte from *Festuca parvigluma* Steud. plants grown in Nanjing Jiangsu and Hangzhou Zhejiang, reported in 2009. Phylogenetic evidences supported that this species should be an interspecific hybrid of ETC (*E. typhina* clade) and *E. yangzii*, and mycological characters, phylogenetic evidences and host specificity indicated *N. sinofestucae* was significantly different from *N. sinicum*(Chen et al., 2008; 2009). In recent studies, a hybrid obtained from *Roegneria canina* (L.) Nevski. grown in Jilin was identified as *N. sinicum*, distribution in Jilin now presents the highest record of latitude of *N. sinicum* (in submission), and also indicates a possibility of distribution in Korean peninsula.

In phylogenetic and karyotic analyses, most epichloë endophytes are significantly different from each other (Tsai et al., 1994; Craven et al., 2001; Moon et al., 2004). They separated into 3 major clades: the clade of *E. typhina* (ETC) including *E. typhina*, *E. sylvatica* and *E. clarkii*, the clade of *E. festucae* (EFC) including *E. baconii* and *E. amatillans*, the clade of *E. brimocola-E. yangzii* complex (EBY) including *E. brimocola*, *E. yangzii*, *E. elymi*, *E. glyceriae* and *E. brachyelytri*. Most asymptomatic *Neotyphodium* spp. were known to be interspecific hybrids among *Epichloë* spp., resulting from karyotic and molecular phylogenetic evidences (Figure). In phylogenetic analyses based on DNA fragments of *tefA* and *tubB*, Chinese native epichloë endophyte species significantly separated into distinct clades, supporting the distinct species. *Neotyphodium* endophytes inhabited in grasses grown in east parts of China were possibly originated from hybridizations between a member of ETC and a member of EBY (Chen et al., 2009; Ji et al., 2009; Kang et al., 2009). This is significantly different from *Neotyphodium* endophytes indigenous in Americas, Europe and Ocean (Schardl et al., 2004; Gentile et al., 2005; Moon et al., 2004; 2007; Iannone et al., 2009). On the other hand, although most Chinese native hybrid endophytes were considered to be progenies of members of ETC and EBY, but presently we still do not have any information on the presence of any ETC member in China (Chen et al., 2009; Ji et al., 2009; Kang et al., 2009). Further survey should be necessary to find this hybridization parent, and efforts for finding this hybridization parent is undergoing in Nanjing.

Epichloë/Neotyphodium complexes have significant resistances to many kinds of insects due to feeding deterrent mediated by several alkaloid compounds produced by the endophytic fungi (Baltz, 2006; Bergmann et al., 2007; Caboche et al., 2007; Dohren, 2009). Peramine, ergopeptines and an unusual pyrrolopyrazines are encoded by modularized non-ribosomal peptide synthetase genes (NRPS genes). NRPS genes are highly conserved in some in several modules, and primers were designed for detections of NRPS genes (Hutchinson, 2003; Fischbach et al., 2007). In our study, we detected 33 fungal samples obtained from 9 genera of host plants by applied nested PCR, and demonstrated that *lpsA* responsible to ergopeptines biosynthesis was detected to be absent in all of 33 isolates, indicating these Chinese native endophytes might be unable to biosynthesis ergopeptines, which is harmful toxic to herbivories including cattle. Adversely, NRPS8 responsible to peramine biosynthesis was identified in almost all the 33 isolates, indicating a potential production of peramine, the most effective insect feeding deterrent produced by epichloë endophytes (Table 2).

Presently, biological and ecological functions of some Chinese native epichloë endophytes were tested both in green houses and in fields (Yu et al., 2009). It will be very valuable if we can have some joint projects with Korean scientists for Asian native epichloë endophytes.

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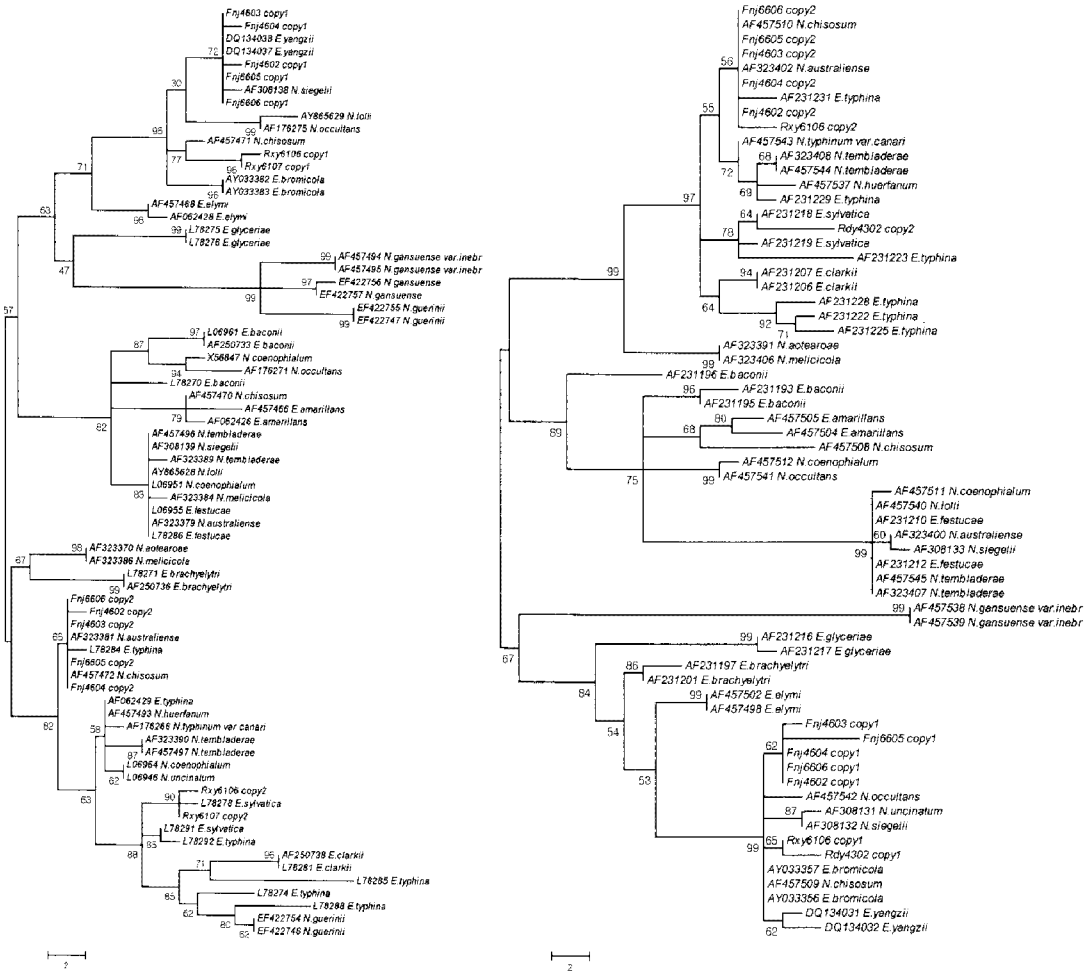

Fig. *tubB* and *tefA* phylograms of *Neotyphodium* spp. and *Epichloë* spp.based on maximum parsimony (MP). Numbers (>60%) at branches are the percentage of trees containing the corresponding clade based on 500 bootstrap replications. *tubB* MP tree shown: tree length = 93 steps; consistency index = 0.860; retention index = 0.964; rescaled consistency index = 0.829. *tefA* MP tree shown: tree length = 192 steps; consistency index = 0.864; retention index = 0.966; rescaled consistency index = 0.834.

Table 1. Twenty events in researches on endophyte bioresources in Chinese or Japanese native grasses

Year	Authors	Country	Endophyte and its hosts	Remarks
1991	Wilkinson *et al*	USA	Endophytic fungi from a *Hordeum* germplasms collected from China	not identified
1993	Miles *et al*	NZ	An *Acremonium*–like endophyte from *Achnatherum inebrians* grown in China	Identified in 2008
1993	Li B. *et al*	China, USA	Endophytic fungi in 3 grasses including *Hordeum* spp.	not identified
1997	Yu *et al.*	Japan	An *Acremonium* endophyte from *Poa pratensis* grown in Japan	not identified
1997	Wang *et al.*	Japan	An *Acremonium* endophyte in Japanese native *Agropyron kamoji* grown in Japan	not identified
1999	Imada *et al.*	Japan	An *Acremonium* endophyte from *Poa trivialis* grown in Japan	Patent, not identified
2000	Wang *et al.*	China*	Epichloë endophytes in many many grassesnative to east part of China	not identified
2002	Nan *et al.*	China	Epichloë endophytes in many grasses native to northern part of China	not identified
2003	Tajimi *et al.*	Japan	An *Epichloë* endophyte from plants of *Brachypodium sylvaticum* grown in Japan	not identified
2003	Wang *et al.*	China*	*Neotyphodium* endophytes in grasses grown in severe saline soils in China	not identified
2003	Li C. *et al.*	China	*Neotyphodium gansuense* Li et al. from *A. inebrians* grown in Northwest of China	First Asian *Neotyphodium*
2006	Li W. *et al.*	China*	*Epichloë yangzii* Li et Wang from *Roegneria* plants grown in Yangtze River basin	First Asian *Epichloë*
2008	Moon *et al.*	NZ	*N. gansuense* var. *inebrians* Moon et al. from *A. sibricum* grown in Northwest of China	
2008	Li C *et al.*	China	An *Epichloë* endophyte from *Dactylis glomerata* grown in west of China	Not identified
2009	Kang *et al.*	China*	*Neotyphodium sinicum* Wang, Ji et Kang from *Roegneria* plants	Widely distributed
2009	Ji *et al.*	China*	*N. stromatolongum* Ji, Zhan et Wang harbored in infertile *Calamagrostis epigeios*	
2009	Chen *et al.*	China*	*N. sinofestucae* Chen, Ji et Wang from *Festuca parvigluma*	
?	Han *et al.*	China*	A new species from *Bromus* spp. in submission	in submission
?	Han *et al.*	China*	*N. sinofestucae* Chen, Ji et Wang from another *Festuca* sp.	in submission
?	Zhang *et al.*	China*	*N. sinicum* Wang, Ji et Kang from *Roegneria canina* plants grown in Jilin	in submission

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