

研究论文

内蒙古中东部草原羽茅*Epichlo*属内生真菌的分布及rDNA-ITS序列系统发育

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摘要 对内蒙古中东部地区分布的羽茅6个地理种群的染菌率进行了调查, 采集种子并从中分离得到不同形态型的内生真菌, 选取其中的19株进行rDNA-ITS片段的扩增、克隆、测序和系统发育分析。结果表明: (1) 6个样地羽茅种群内生真菌感染率除西乌旗为96.7%外, 其他5个样地均为100%, 表明内生真菌侵染羽茅并非偶然现象, 二者之间存在一种稳定的共生关系。(2) ITS和5.8S序列得到的N-J树显示, 相对于*Epichlo*属的其他参考菌株, 不同地理种群羽茅中的内生真菌聚为一类, 形成一个具有97%支持强度的分支。由此推测, 不同地理种群羽茅中的内生真菌具有相同的起源点。(3) 结合形态观察结果和rDNA-ITS序列分析结果可以看出, 羽茅内生真菌种群的优势种亲缘关系较近, 可能起源于同一种内生真菌; 但由于其地理分布广、气候差异大、群落类型差别也较大, 从而造成不同地理种群内生真菌形态上的分化以及种群间明显的遗传分化和较高的遗传多样性。

关键词 [羽茅](#); [内生真菌](#); [系统发育](#); [rDNA-ITS](#)

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Distribution and rDNA-ITS sequence analysis of *Epichlo* e ndophyte symbiosis with *Achnatherum sibiricum* in mid- and eastern Inner Mongolia Steppe

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Abstract The complex functions of fungal endophytes have received intensive study during the past three decades because of their roles in production loss of livestock and enhancement of biotic and abiotic stress resistances in hosts. Most of these studies focused on cultivated grasses, but little is known about the distribution and mechanisms of endophytes in native grass populations. Surveys of grass species associated with *Neotyphodium* and *Epichloë* fungal endophytes made in native grasslands of northern China suggested that endophytes are widely distributed in natural grass populations. Among them, *Achnatherum sibiricum* was found to associate with fungal endophytes in all sites investigated. *Ach. sibiricum* is a perennial, sparse bunch grass that inhabits temperate steppe in China. This grass can be found in different habitats such as meadows, typical steppes and forests. Its distribution may be largely determined by precipitation, while it is usually a comp

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anion species in communities and it sometimes can become the dominant species. Untill now, no toxicosis problems in grazing animals have been reported in pastures containing *Ach. sibiricum*. Six natural populations (IMGERS-CAS, *Leymus chinensis* plot of the IMGERS-CAS, Xi Ujimqin Qi, Hulingol, Arshan, Hailar) of *Ach. sibiricum* in mid- and eastern Inner Mongolia Steppe of varying distances from each other were chosen. Locations and descriptions of sampling sites were recorded. Infection frequency of fungal endophytes was detected and seeds from 30 *Ach. sibiricum* individuals were collected. Fungal endophytes of different morphologies were isolated from seeds and at each site, strains of different morphologies were used as internal transcribed spacers of the nuclear ribosomal DNA (rDNA-ITS) sequence analysis. Regions of rDNA-ITS were amplified, cloned, sequenced and submitted to GenBank. Furthermore, phylogenetic analysis was conducted with additional sequences conserved in GenBank.

Fungal endophytes were found at high frequencies in all investigated local populations regardless of their location, elevation, annual precipitation, mean temperature and vegetation types, implying a systemic association between native grasses and fungal endophytes. In plants which have seed-transmitted endophytes, high infection frequency suggests a selective advantage compared with uninfected plants. This may be due to increased resistance to pathogens or herbivores. Another possible factor is that endophyte infection enhanced plant's resistance to an abiotic stress, such as drought or fire.

Nineteen strains from six populations were used in rDNA-ITS sequence analysis. They were IB from IMGERS-CAS; LB, LC, LL from *Leymus chinensis* plot of the IMGERS-CAS; XA, XB, XD, XE, XF, XG from Xi Ujimqin Qi; HA, HE from Hulingol; AA, AB, AC, AD from Arshan and EA, EB, EC from Hailar. Besides LC, LL and XE, which were pathogens belonged to Ascomycota but not *Epichloa*, there were only five informative sites in the alignment of 16 rDNA-ITS sequences and there were no correlation between differences of sequences and geographical distances. Among them IB, LB, AC and EB have the same sequences, which is consistent with the results of morphological observation. Results of Maximum parsimony tree and N-J tree based on ITS & 5.8S sequences of 16 fungal strains showed that all *Epichloa* symbionts with *Ach. sibiricum* were clustered into one taxonomic group with 97% bootstrap support compared to other reference endophyte isolates used in this analysis. Therefore we propose that all *Epichloa* symbionts share the same evolutionary origin. Their diverse morphologies and high genetic variations among different populations are attributed to broad distribution of *Ach. sibiricum* and differences in climate and plant community.

Key words [_ *Achnatherum sibiricum* _ endophytes _ phylogeny _ rDNA-ITS](#)

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