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Finding the core fungal species affecting bioactive compound production in *Gastrodia sp.* by comparative metagenomics

Ruey-Hua Lee, Yo-Chin Lu, Shang-Ting Lin, En-Tse Chang, Tzen-Yuh Chiang and Yi-Min Chen National Cheng Kung University, Taiwan

Pastrodia sp. is myco-heterotrophic orchid found mainly in Asia. Large tuber species such as G. elata (Tianma) and G. Gesamoides are traditionally used as Han remedy and food, respectively. Scientific studies using animal models also show the bioactive compounds from G. elata provide anti-convulsion, anti-oxidation, neuroprotection, memory improvement and anti-inflammation effects. Our long term aim is to identify small peptides that play crucial roles in Gastrodia sp. and microbial interaction and in affecting the bioactive compounds production. Metagenomics is a sequence-based tool for analyzing microbial communities in the environmental samples on a huge scale regardless of the ability of member organisms to be cultured in the laboratory. In this work, we have compared fungal diversities of three native Gastrodia sp. with cultivated G. elata and between Gastrodia samples with different gastrodin contents using meta-genomics approaches. We first compared resolution of six primer pairs designed from nuclear rRNA barcode region including large subunit 28S rRNA (nuLSU), small subunit 18S rRNA (nuSSU) and internal transcribed spacer (ITS). Two primer pairs were used in each of these regions. Split-plot MANOVA and multiple comparisons show distinct fungal abundance and diversity between different species and primer pairs used. This suggests that multiple primer pairs designed in the same and different gene regions should be used for amplicon libraries preparation in metagenomics studies. Comparative metagenomics also carried out for the purpose of identifying the core microbiome in G. elata that affecting bioactive compounds production using samples that show different levels of gastrodin after postharvest processing and wild samples. Our preliminary data suggests that some candidate species of saprophytic fungi may play significant role in induction of bioactive compound production.

shanhua@mail.ncku.edu.tw