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The complete mitochondrial genome of huperzine A-producing endophytic fungus *Penicillium polonicum*

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ABSTRACT

Huperzine A-producing fungus *Penicillium polonicum* Hy4 (CCTCC No.M2010086) was isolated from *Huperzia serrata* (Thunb) Trev. The complete mitochondrial genome of *P. polonicum* is 28 192 bp in length, containing 15 protein-encoding genes, 27 tRNA genes and two rRNA genes. The whole mitogenome is high in AT content (74.40%) and low in GC content (25.60%). The mitochondrial gene order and arrangement of *P. polonicum* are identical to those of other *Penicillium*. Phylogenetic analysis based on 14 concatenated protein-encoding genes showed that *P. polonicum* was close to *P. solitum*. This study reports the complete mitogenome of *P. polonicum* for the first time and provides valuable information for further exploration of mitochondrial evolution.

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Evolution; fungi; mitogenome; *Penicillium polonicum*

Penicillium polonicum strain Hy4 (CCTCC No.M2010086) , isolated from *Huperzia serrata* in Shaoyang, Hunan, China (27.22 North, 111.50 East) was found to produce Huperzine A (Hup A) and was deposited in China Center for Type Culture Collection (CCTCC). Hup A is used to treat dementia as a reversible and selective acetylcholinesterase (AChE) inhibitor with low toxicity (Zhang & Tang 2006). Up to now, Hup A is mainly isolated from *H. serrata* which have an extremely long vegetative growth cycle and low Hup A content (Ma et al. 2005). Endophytic fungi are one of the most creative groups of high-value secondary metabolite producers (Venugopalan & Srivastava 2015).

Initial taxonomic evaluation of *P. polonicum* Hy4 was made based on ITS sequence (Makimura et al. 2001). The mitogenome was sequenced by Illumina Hiseq 2000 and assembled by Allpaths-LG (Gnerre et al. 2011), with two gaps filled by PCR amplification. It was a circular-mapping DNA molecule of 28 192 bp with a low GC content of 25.60% (Genbank accession number: KU530219). The mitogenome contained 15 conserved protein-encoding genes (13 728 bp, 48.69% of the mitogenome) that were discovered by searching NCBI NR database using Exonerate (Slater & Birney 2005; Lin et al. 2015). Those genes encoded ATP synthase subunits (*atp6*, *atp8* and *atp9*), cytochrome oxidase subunits (*cox1*, *cox2* and *cox3*), apocytochrome b (*cob*), NADH dehydrogenase subunits (*nad1*, *nad2* and *nad3*, *nad4*, *nad4L*, *nad5* and *nad6*) and ribosomal protein (*rps3*). All genes were located on one strand and apparently transcribed in one direction. Additionally, 27 tRNA genes were predicted by tRNAscan-SE (Lowe & Eddy 1997) and the large/small subunits ribosomal RNA genes (*rns*, *rnl*) were identified by searching against Rfam database (Burge et al. 2013). Similar to many filamentous fungi, *P. polonicum* mitochondrial tRNA genes were organized into two dense gene clusters (KGGDSWISP, TEVMMLAFLQM), which were flanked by *cox3*, *rnl* and *cox1* (Sun et al. 2011; Eldarov

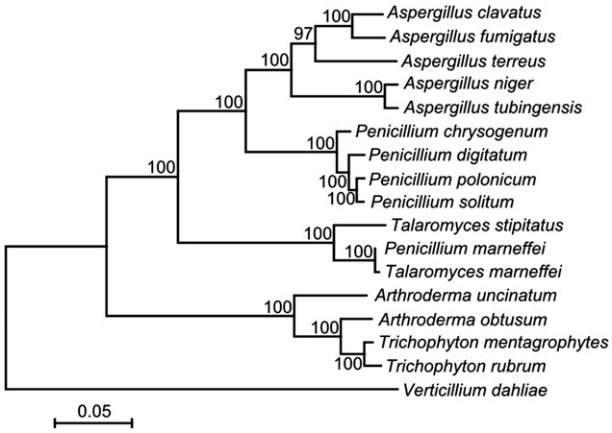

Figure 1. Phylogenetic relationship between *P. polonicum* and other 16 fungal mitogenomes. The maximum likelihood tree was generated by MEGA v6.06 (Tamura et al. 2013) based on 14 concatenated protein-coding genes (*nad1-6*, *nad4L*, *atp6*, *atp8-9*, *cob* and *cox1-3*). *Verticillium dahliae* was used as outgroup. The model for phylogenetic analysis was GTR + G+I with bootstrap value of 1000. The sequence data for these 17 fungi with complete mitochondrial genomes were used: *Aspergillus clavatus* (JQ354999), *Aspergillus fumigatus* (JQ346807), *Aspergillus terreus* (JQ355001), *Aspergillus niger* (NC_007445), *Aspergillus tubingensis* (NC_007597), *Penicillium chrysogenum* (JQ354996), *Penicillium digitatum* (NC_015080), *Penicillium polonicum* (KU530219), *Penicillium solitum* (NC_016187), *Talaromyces stipitatus* (JQ354994), *Penicillium marneffei* (JQ354997), *Talaromyces marneffei* (NC_005256), *Arthroderma uncinatum* (NC_012828), *Arthroderma obtusum* (NC_012830), *Trichophyton mentagrophytes* (NC_012826), *Trichophyton rubrum* (NC_012824), *Verticillium dahliae* (DQ351941).

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et al. 2012; Joardar et al. 2012). The comparison of *trn* distribution in the mitogenomes showed that the *trn* order in *Penicillium* was relatively conserved in contrast to that in *Aspergillus*.

The codon frequency analysis showed that a total of 56 codons were used for transcription, with the absence of ACC, CCC, CGA, CGC, CGG, CTC, GGC and TCG. All protein-encoding sequences started with the typical ATG codon and ended with TAA as the stop codons except for *nad6* and *cox3* (TAG). AT-rich codons were abundant, up to 82.36%, reflecting the high AT content of the *P. polonicum* mitogenome. The most used codon is TTA for Leu and followed by ATA for Ile. The ratio of codons encoding hydrophobic amino acids (Met, Trp, Phe, Val, Leu, Ile, Pro, Ala) was 54.74%, reflecting the hydrophobic nature of respiratory membrane complexes. A maximum likelihood tree generated by 14 protein-coding genes from 17 fungal strains (Figure 1) suggested the closest relationship between *P. polonicum* and *P. solitum*. Moreover, *Penicillium* was closely related to *Aspergillus*, except *Penicillium marneffei*, which was positioned on a distinct branch. This observation was identical with the earlier study based on nuclear genome (van den Berg et al. 2008).

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Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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References

Burge SW, Daub J, Eberhardt R, Tate J, Barquist L, Nawrocki EP, Eddy SR, Gardner PP, Bateman A. 2013. Rfam 11.0: 10 years of RNA families. Nucleic Acids Res. 41: D226–D232.

Eldarov MA, Mardanov AV, Beletsky AV, Dzhavakhiya VV, Ravin NV, Skryabin KG. 2012. Complete mitochondrial genome of compactin-producing fungus *Penicillium solitum* and comparative analysis of Trichocomaceae mitochondrial genomes. FEMS Microbiol Lett. 329:9–17.

Gnerre S, Maccallum I, Przybylski D, Ribeiro FJ, Burton JN, Walker BJ, Sharpe T, Hall G, Shea TP, Sykes S, et al. 2011. High-quality draft assemblies of mammalian genomes from massively parallel sequence data. Proc Natl Acad Sci USA. 108:1513–1518.

Joardar V, Abrams NF, Hostetler J, Paukstelis PJ, Pakala S, Pakala SB, Zafar N, Abolude OO, Payne G, Andrianopoulos A, et al. 2012. Sequencing of mitochondrial genomes of nine *Aspergillus* and *Penicillium* species identifies mobile introns and accessory genes as main sources of genome size variability. BMC Genomics. 13:698.

Lin R, Liu C, Shen B, Bai M, Ling J, Chen G, Mao Z, Cheng X, Xie B. 2015. Analysis of the complete mitochondrial genome of *Pochonia chlamydosporia* suggests a close relationship to the invertebrate-pathogenic fungi in Hypocreales. BMC Microbiol. 15:5.

Lowe TM, Eddy SR. 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res. 25:955–964.

Ma X, Tan C, Zhu D, Gang DR. 2005. Is there a better source of huperzine A than *Huperzia serrata*? Huperzine A content of Huperziaceae species in China. J Agric Food Chem. 53:1393–1398.

Makimura K, Hanazawa R, Takatori K, Tamura Y, Fujisaki R, Nishiyama Y, Abe S, Uchida K, Kawamura Y, Ezaki T, et al. 2001. Fungal flora on board the Mir-Space Station, identification by morphological features and ribosomal DNA sequences. Microbiol Immunol. 45:357–363.

Slater GS, Birney E. 2005. Automated generation of heuristics for biological sequence comparison. BMC Bioinformatics. 6:31.

Sun X, Li H, Yu D. 2011. Complete mitochondrial genome sequence of the phytopathogenic fungus *Penicillium digitatum* and comparative analysis of closely related species. FEMS Microbiol Lett. 323:29–34.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol. 30:2725–2729.

Van Den Berg MA, Albang R, Albermann K, Badger JH, Daran JM, Driessen AJ, Garcia-Estrada C, Fedorova ND, Harris DM, Heijne WH, et al. 2008. Genome sequencing and analysis of the filamentous fungus *Penicillium chrysogenum*. Nat Biotechnol. 26:1161–1168.

Venugopalan A, Srivastava S. 2015. Endophytes as *in vitro* production platforms of high value plant secondary metabolites. Biotechnol Adv. 33:873–887.

Zhang HY, Tang XC. 2006. Neuroprotective effects of huperzine A: new therapeutic targets for neurodegenerative disease. Trends Pharmacol Sci. 27:619–625.