



Complete mitochondrial genome sequence of Afla-Guard[®], commercially available non-toxicogenic *Aspergillus flavus*

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ABSTRACT

Afla-Guard[®] is a commercial non-toxicogenic *Aspergillus flavus* strain used to decrease aflatoxin contamination level in field. Its mitochondrial genome was sequenced, showing that its length is 29,208 bp with typical configuration of *Aspergillus* mitochondrial genome. 17 SNPs and 27 INDELS were identified by comparing with previous *A. flavus* mitochondrial genome. Phylogenetic trees present that *A. flavus* of Afla-Guard[®] was clustered with the previous *A. flavus* mitochondrial genome.

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Aspergillus flavus; Afla-Guard[®]; mitochondrial genome; *Aspergillus*; Ascomycota

Afla-Guard[®] is a commercial product to decrease aflatoxin contamination level for improving quality of corns and peanuts (Wu et al. 2008; Durham et al. 2010; Dorner and Lamb 2006). This product contains *Aspergillus flavus* without aflatoxin gene cluster usually found in *A. flavus* (Abdel-Hadi et al. 2012). It can be used to understand phylogenetic position of nontoxic *A. flavus*. In addition, two mitochondrial genomes of *A. flavus* present that two genomes show different phylogenetic positions (data not shown), requiring additional mitochondrial genomes of *A. flavus* for clarifying this problem.

DNA of Afla-Guard[®] purchased from Syngenta was extracted using the optimized protocol (Lee et al. 2017). It was originally isolated from peanut seed at the USDA National Peanut Research Laboratory (Georgia, USA) in 1991 (NRRL 21882 in Agricultural Research Service Culture Collection). Raw data generated by HiSeq2500 and *de novo* assembly was conducted by Velvet 1.2.10 (Zerbino and Birney 2008). Gap filling was done by SOAPGapCloser 1.12 (Zhao et al. 2011) after confirming each base using BWA 0.7.17 and SAMtools 1.9 (Li et al. 2009; Li 2013). Geneious R11 11.0.5 (Biomatters Ltd, Auckland, New Zealand) was used to annotate its mitogenome by comparing with those of *A. flavus* (JQ355000; Joardar et al. 2012).

The length of Afla-Guard[®] *A. flavus* mitogenome (GenBank accession is MT335777) is 29,208 bp, which is 3 bp longer than that of previously reported *A. flavus* (JQ355000). It is the 8th shortest mitogenome among 23 available *Aspergillus* mitogenomes (Park et al., in preparation). Numbers of PCGs,

tRNAs, and rRNAs are 17, 27, and 2, respectively a typical configuration of *Aspergillus* mitogenomes.

Seventeen SNPs and 27 INDELS were identified as intra-specific variation on two *A. flavus* mitochondrial genomes. Two non-synonymous SNPs (nsSNPs) were identified in *NAD1*, two nsSNPs and three INDELS were found in hypothetical protein, and one nsSNP was in *NAD4*. Two synonymous SNPs (sSNPs) were found in *COX1* and another two sSNPs were in *NAD5*. The remaining SNPs and INDELS were in intergenic space. Numbers of intraspecific variations are relatively large in comparison to those of *Aspergillus oryzae*, *Aspergillus terreus*, and *Penicillium digitatum* (Park et al., in preparation).

Sequence alignments of ten conserved genes from twelve *Aspergillus* and one *Penicillium* mitogenomes (Juhász et al. 2008; Futagami et al. 2011; Sun et al. 2011; Joardar et al. 2012; Zhao et al. 2012; Park, Kwon, Huang, et al. 2019; Park, Kwon, Zhu, Mageswari, Heo, Han, et al. 2019; Park, Kwon, Zhu, Mageswari, Heo, Kim, et al. 2019) including that of Afla-Guard[®] and one *Podospira* mitochondrial genome (Cummings et al. 1990) as an outgroup were calculated by MAFFT 7.450 (Katoh and Standley 2013) and concatenated. The neighbour-joining (10,000 bootstrap repeats) and maximum-likelihood (1,000 bootstrap repeats) phylogenetic trees were constructed using MEGA X (Kumar et al. 2018). Phylogenetic trees showed that two *A. flavus* mitochondrial genomes were clustered in one clade together with *A. oryzae* (Figure 1). In addition, another *A. flavus* mitochondrial genome (NC_026920) was clustered with *A. nidulans* (Figure 1), addressing its species identification.

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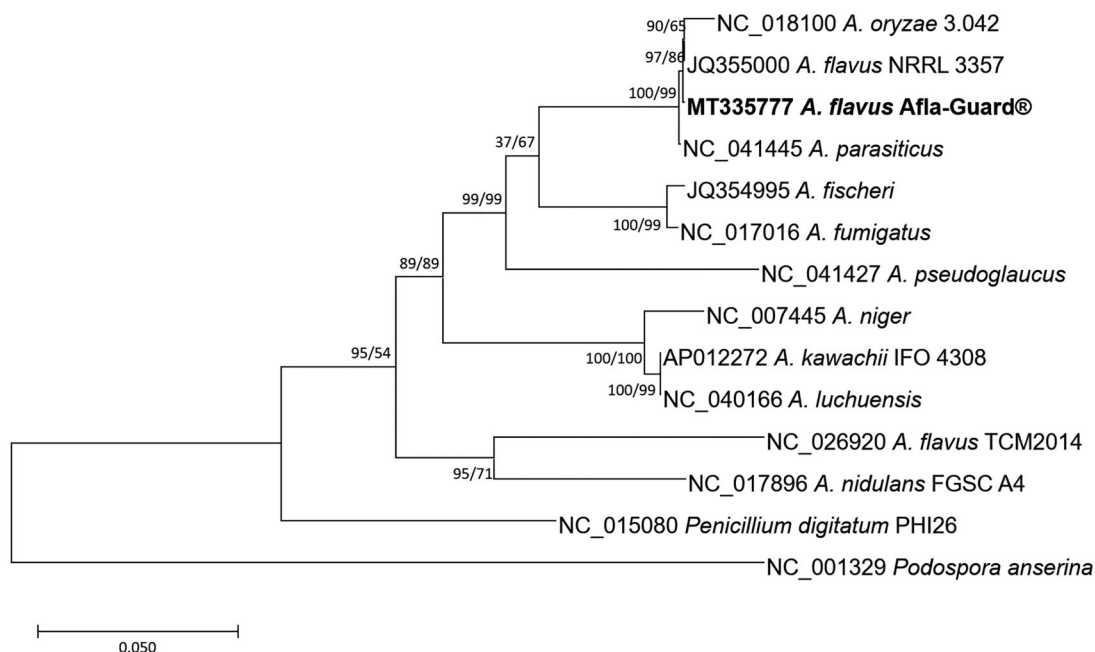


Figure 1. Maximum-likelihood (bootstrap repeat is 1000) and neighbour-joining (bootstrap repeat is 10,000) phylogenetic trees of twelve *Aspergillus*, one *Penicillium* mitochondrial genome, and *Podospira* mitochondrial genome as an outgroup: *Aspergillus flavus* (MT335777 in this study, JQ355000, and NC_026920), *Aspergillus oryzae* (NC_018100), *Aspergillus parasiticus* (NC_041445), *Aspergillus fischeri* (JQ354995), *Aspergillus fumigatus* (NC_017016), *Aspergillus pseudoglaucus* (NC_041427), *Aspergillus niger* (NC_007445), *Aspergillus kawachii* (AP012272), *Aspergillus luchuensis* (NC_040166), *Aspergillus nidulans* (NC_017896), *Penicillium digitatum* (NC_015080), and *Podospira anserina* (NC_001329). Phylogenetic tree was drawn based on maximum-likelihood phylogenetic tree. The numbers above or below branches indicate bootstrap support values of maximum likelihood and neighbour-joining phylogenetic trees, respectively.

Disclosure statement

The authors declare that they have no competing interests.

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Data availability statement

The sequence can be accessed via accession number MT335777 in NCBI GenBank. <https://www.ncbi.nlm.nih.gov/genbank/>.

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