

Genome Sequence of *Aureobasidium pullulans* AY4, an Emerging Opportunistic Fungal Pathogen with Diverse Biotechnological Potential

Giek Far Chan, a,b Hasima Mustafa Bamadhaj,b Han Ming Gan,c and Noor Aini Abdul Rashida,b

Nanoporous Materials for Biological Application Research Group (NAMBAR), Sustainability Research Alliance, Universiti Teknologi Malaysia, Johor Bahru, Johor, Malaysia^b; Department of Biological Sciences, Faculty of Biosciences and Bioengineering, Universiti Teknologi Malaysia, Johor Bahru, Johor, Malaysia^b; and ScienceVision SB, Setia Alam, Shah Alam, Selangor, Malaysia^c

Aureobasidium pullulans AY4 is an opportunistic pathogen that was isolated from the skin of an immunocompromised patient. We present here the draft genome of strain AY4, which reveals an abundance of genes relevant to bioindustrial applications, including biocontrol and biodegradation. Putative genes responsible for the pathogenicity of strain AY4 were also identified.

he fungus Aureobasidium pullulans (de Bary) G. Arnoud, known as the "black yeast," was first described as a new fungal species under the name Dermatium pullulans by de Bary in 1866. However, in 1910, a new taxonomic combination under A. pullulans was proposed (7). A. pullulans has been regarded as having various biotechnology and environmentrelated applications, such as production of extracellular polymeric substances, especially pullulan, production of hydrolytic enzymes, including amylases, proteases, esterases, pectinases, xylanases, and mannanases (3, 8), and as a biocontrol agent for plants (9). Various forms of this polymorphic fungus have been ubiquitously isolated from natural environments (11). However, the fungus has been increasingly associated with human cutaneous and invasive infections (1). Locally, a pathogenic strain of A. pullulans, designated AY4, was coisolated with Candida orthopsilosis from an immunocompromised patient suffering from primary aldosteronism with persistent cutaneous infection (2). The draft genome of strain AY4 was determined and annotated to gain further insight into its biotechnological properties and pathogenicity.

The draft genome sequence of strain AY4 was determined using the Genome Analyzer IIx system (Illumina). The 100-bp paired-end reads were assembled de novo using the CLC Genomics Workbench software program (CLC bio, Denmark) into 515 contigs of 46-fold coverage with an average length of 51,889 bp. The N_{50} length is 223,969 bp, and the longest contig is 857,588 bp. The draft genome sequence of strain AY4 contains 26,722,706 bp, with an average GC content of 49.82%. A total of 10,288 putative open reading frames (ORFs) were predicted using the software program GeneMark-ES (10). Based on Blast2GO (4) annotation, 8,101 ORFs have at least one BLAST hit in the current NCBI public database. A total of 261 tRNAs were identified using the tRNAscan-SE 1.3 server (6). As analyzed using the RNAmmer 1.2 server (5), strain AY4 possesses 19 copies of 8S rRNA and a copy (each) of 28S and 18S rRNA.

The draft genome of strain AY4 reveals the rich presence of genes coding for commercially important enzymes, including amylases, glucoamylases, glucose oxidases, xylanases, polygalacturonases, phytases, alcohol dehydrogenases, cellulases, xylose reductase, dextranase, pullulanase, trehalose synthase, and urease. In addition, the presence of alkaline serine protease, glucanase, and

chitinase, as well as aureobasidin biosynthesis genes, verifies the well-reported role of *A. pullulans* in its antagonism against pytopathogenic fungi. In addition, genes coding for phenol 2-monooxygenases and catechol dioxygenases are identified in the draft genome of strain AY4, suggesting its potential in the biodegradation of aromatic pollutants. Interestingly, strain AY4 also possesses a gene coding for depolymerase, a potential enzyme catalyzing the biodegradation of plastic.

Despite the significant biotechnological potentials of strain AY4, its pathogenicity should not be overlooked. Based on the draft genome, strain AY4 contains genes implicated in biofilm formation and a number of important virulence factors, including lipases, phospholipases, proteases, and beta-lactamases. The KEGG pathways derived from the Blast2GO annotations will provide useful insights for the future development of antifungal drugs against pathogenic *A. pullulans*.

Nucleotide sequence accession number. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession no. AMCU000000000. The version described in this article is the first version, accession no. AMCU01000000.

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REFERENCES

- 1. Chan GF, Puad MSA, Chin CF, Rashid NAA. 2011. Emergence of *Aureobasidium pullulans* as human fungal pathogen and molecular assay for future medical diagnosis. Folia Microbiol. 56:459–467.
- 2. Chan GF, Puad MSA, Rashid NAA. 2011. Candida orthopsilosis and Aureobasidium pullulans: rare fungal pathogens causing persistent skin infection. Insight Infect. Dis. 1:1–4.
- 3. Chi Z, et al. 2009. Bioproducts from *Aureobasidium pullulans*, a biotechnologically important yeast. Appl. Microbiol. Biotechnol. 82:793–804.

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- 4. Conesa A, et al. 2005. Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics 21: 3674–3676.
- Lagesen K, et al. 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res. 35:3100-3108.
- 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.
- 7. Matsumoto T, Padhye AA, Ajello L. 1987. Medical significance of the so-called black yeasts. Eur. J. Epidemiol. 3:87–95.
- 8. Ravella SR, et al. 2010. Extracellular polysaccharide (EPS) production by
- a novel strain of yeast-like fungus *Aureobasidium pullulans*. Carbohydr. Polym. **82**:728–732.
- 9. Schmid F, Moser G, Muller H, Berg G. 2011. Functional and structural microbial diversity in organic and conventional viticulture: organic farming benefits natural biocontrol agents. Appl. Environ. Microbiol. 77: 2188–2191.
- 10. Ter-Hovhannisyan V, Lomsadze A, Chernoff YO, Borodovsky M. 2008. Gene prediction in novel fungal genomes using an ab initio algorithm with unsupervised training. Genome Res. 18:1979–1990.
- 11. Zalar P, et al. 2008. Redefinition of *Aureobasidium pullulans* and its varieties. Stud. Mycol. 61:21–38.

1420 ec.asm.org Eukaryotic Cell