Certificate of Analysis for NR-43222

Cryptococcus gattii, Strain Alg254

Catalog No. NR-43222

Product Description:

Cryptococcus gattii (C. gattii), strain Alg254 is a mutant of strain R265 where the basidiomycete white collar 2 (BWC2) gene has been replaced with a cassette conferring resistance to nourseothricin. NR-43222 was produced by inoculation of BEI Resources seed lot 61632120 into Yeast Mold agar, which was grown for 3 days at 25°C in an aerobic atmosphere. The agar growth was harvested with 10% glycerol to produce this lot.

Lot: 70066655 **Manufacturing Date: 15MAR2024**

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TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology 3 days at 25°C in an aerobic atmosphere on Yeast Mold agar	Report results	Globose-to-subglobose; slightly pyriform; no pseudohyphae observed (Figure 1)
Colony morphology 3 days at 25°C in an aerobic atmosphere on Yeast Mold agar	Report results	Circular, butyrous and cream (Figure 2)
VITEK® MS (MALDI-TOF)	*C. gattii*	*C. gattii* (99.9%)
Canavanine-glycine-bromthymol blue (CGB) differential medium[1] 2 days at 25°C in an aerobic atmosphere	Blue *(C. gatti)*	Blue *(C. gatti)*
Genotypic Analysis		
Sequencing of partial 18S ribosomal RNA (rRNA) gene, internal transcribed spacer (ITS) 1, 5.8S rRNA gene, ITS 2, partial 26S rRNA (~520 base pairs)	≥ 99% sequence identity to *C. gattii* (GenBank: FJ914888.1)	100% sequence identity to *C. gattii* (GenBank: FJ914888.1)[2]
Sequencing of 28S rRNA gene (~ 620 base pairs)	≥ 99% sequence identity to *C. gattii* (GenBank: KC171326.1)	100% sequence identity to *C. gattii* (GenBank: KC171326.1)[2]
Purity		
Nutrient broth with 0.1% Yeast Extract at 25°C 3 days in an aerobic atmosphere	No bacterial growth	No bacterial growth
Nutrient broth with 0.1% Yeast Extract at 37°C 3 days in an aerobic atmosphere	No bacterial growth	No bacterial growth
Viability (post-freeze) 3 days at 25°C in an aerobic atmosphere on Yeast Mold agar	Growth	Growth

[1]CGB medium differentiates *C. gattii* from *C. neoformans* based on the ability of *C. gatti* isolates to grow in the presence of L-canavanine and to assimilate glycine as a sole carbon source, resulting in a blue color. *C. neoformans* isolates will show yellow to light-green on CGB medium. (McTaggart, L., et al. "Rapid Identification of *Cryptococcus neoformans* var. *grubii*, *C. neoformans* var. *neoformans*, and *C. gattii* by Use of Rapid Biochemical Tests, Differential Media, and DNA Sequencing." J. Clin. Microbiol. 49 (2011): 2522-2527. PubMed: 21593254.)

[2]Also consistent with *Cryptococcus* species *gattii*, *deuterogattii*, *tetragattii neoformans* and *bacillisporus*

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Figure 1: Cellular Morphology

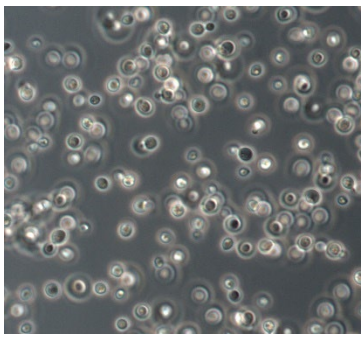
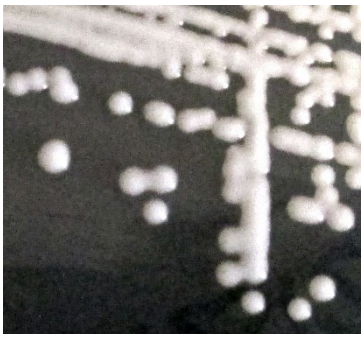


Figure 2: Colony Morphology



/Sonia Bjorum Brower/
Sonia Bjorum Brower 15 JAN 2025
Technical Manager or designee, ATCC Federal Solutions

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