

Certificate of Analysis for NR-43223

Cryptococcus gattii, Strain Alg268

Catalog No. NR-43223

Product Description: Cryptococcus gattii (C. gattii), strain Alg268 is a complementation mutant of strain Alg254 (the basidiomycete white collar 2 (BWC2) gene was replaced with a nourseothricin cassette). The wild type BWC2 gene was amplified and inserted into a plasmid, which was transformed into Agrobacterium tumefaciens, and transconjugated into C. gatti, strain Alg254.

Lot^{1,2}: 61631852 Manufacturing Date: 29MAR2013

TEST	SPECIFICATIONS	RESULTS
Phenotypic Analysis		
Cellular morphology ³	Report results	Sub-globose to ovoid, single (Figure 1A)
Colony morphology ³	Report results	Smooth, mucoid, entire and cream (Figure 1B)
Canavanine-glycine-bromthymol blue (CGB) differential medium ⁴	Blue (C. gatti)	Blue (C. gatti)
Genotypic Analysis		-
Sequencing of partial 18S rRNA gene, internal transcribed spacer (ITS) 1, 5.8S rRNA gene, ITS 2, partial 28S rRNA (~ 520 base pairs)	Consistent with <i>C. gattii</i>	Consistent with <i>C. gattii</i> ⁵
Sequencing of 26S rRNA gene (~ 620 base pairs)	Consistent with C. gattii	Consistent with <i>C. gattii</i> ⁵
Purity ⁶		
Nutrient broth with 0.1% Yeast Extract at 25°C	No bacterial growth	No bacterial growth
Nutrient broth with 0.1% Yeast Extract at 37°C	No bacterial growth	No bacterial growth
Viability (post-freeze) ²	Growth	Growth

NR-43223, lot 61631852, was produced by the depositor by incubation at 30°C in Yeast Peptone Dextrose medium overnight. The resultant growth was mixed with 30% glycerol to a final concentration of 15% and vialed.

Figure 1: Cellular morphology (A) and colony morphology (B)

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²Quality control testing was performed at BEI Resources.

³2 days at 25°C in an aerobic atmosphere on Yeast Mold agar

⁴35 hours at 27°C in an aerobic atmosphere. 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. 2011 (49): 2522-2527. PubMed: 21593254.]

⁵Also consistent with *C. neoformans*

⁶Clarity of broth was determined by visual inspection after 2 days at 25°C and 37°C in an aerobic atmosphere.



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Date: 24 FEB 2015

Signature:

Title:

Technical Manager, BEI Authentication or designee

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