

Heterokaryon - FGSC #4564 plus vph-1-RIP, A

This strain is deficient in subunit a (100 kDa) of the vacuolar ATPase. It appears to be a complete null and makes no functional enzyme.

A V-ATPase null strain has severe morphological abnormalities. It makes no conidia and few aerial hyphae. On agar plates with Vogel's salts and 2% sucrose it forms a compact, hyphal mat that grows to the edge of a standard petri dish in approximately 4 days at 30 C. In liquid culture it tends to form round clumps of mycelia. Vma-RIP strains are pH-conditional lethals. They will not grow on medium with 20 mM HEPES buffer, pH 7.2 or higher.

The strain is maintained as a heterokaryon with the inositol-requiring helper strain, FGSC 4564. Crosses with this heterokaryon are fertile, but spores that are vma-RIP germinate with very low efficiency (1.0 - 0.1%). Crosses in which both parents are vma-RIP strains are not fertile.

To generate the vma-RIP strain from the heterokaryon, spread conidia on minimal plates (typically sorbose plates). Grow 2 days at 30 C. The colonies that appear will be of three morphological types. Heterokaryons will be vigorous and look like typical "wild-type" colonies. Homokaryotic vma-RIP colonies will be roughly the same diameter as the heterokaryons, but have few aerial hyphae. Most of the growth is on the surface of the agar. A third type, thin wispy colonies may also appear. These are probably inositol-requiring homokaryons of the helper strain because they usually stop growing in a few days. If a vma-RIP colony is transferred to liquid medium it will form a compact, submerged mycelium that takes several days to grow to the surface. A good additional test is to measure for pH-conditional lethality, as described above.