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A mutant of *Metarhizium anisopliae* var. *acridum* with enhanced submerged conidiation

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Summary

An insertional mutant of *Metarhizium anisopliae* is described with enhanced submerged conidiation. In a 500 ml submerged culture, this mutant produces a mean of 4.05×10^8 propagules ml^{-1} from an inoculum of 1×10^6 conidia, where the parental strain accumulates only 3.75×10^4 propagules ml^{-1}.

The mitosporic Hyphomycete fungus, *Metarhizium anisopliae* var. *acridum* is of considerable interest as a specific biocontrol agent for locusts and grasshoppers. A Brazilian isolate, *M. anisopliae* var. *acridum* CG423, was found to cause high mortality in local pest species of grasshoppers and is currently being developed in Brazil as a biological pesticide (Magalhães *et al.* 2001). As part of the further development and improvement of this strain, we previously reported its co-transformation with genes for green fluorescent protein (*egfp*) and resistance to gluphosinate ammonium (*bar*) (Inglis *et al.* 2000). From among these co-transformants, we later observed a strain, CG819, showing abundant submerged conidiation in deep shake-flask cultures. The culture medium of this strain became a deep green color within 96 h, due to the accumulation of pigmented conidia (Figure 1a); producing a mean of 4.05×10^8 propagules ml^{-1} in triplicate flasks (500 ml medium in 1000 ml conical flask). Under the same conditions, the culture medium of the wild-type parental strain, CG423 accumulated a mean of 3.75×10^4 propagules ml^{-1} and another *bar*/*egfp* co-transformant of this strain, CG820, produced 3.5×10^5 propagules ml^{-1}. Under conditions of submerged conidiation, the mycelial phase of CG819 appears to be severely restricted. The cultures appear heterogeneous, where spores appear to be budded directly from other unicellular propagules or be produced from slightly elongated phialide-like structures, which appeared to be free or as branches of stunted hyphae (arrowed, Figure 1b). In contrast, the morphology of CG820 in deep shake flask cultures is wild-type (Figure 1c); appearing as clusters or pellets of elongated, non-pigmented mycelial hyphae.

Slide cultures were set up to observe conidiation within agar. Strain CG819 was found to conidiate readily within the agar layer (Figure 2a). In contrast, conidiation within the agar blocks appeared to be totally suppressed in wild-type parental strain, CG423 and the control co-transformant, CG820 (Figure 2b). No significant differences in the appearance of normal aerial conidiation at the exposed edges of the slide cultures were observed between the three strains. The embedded conidia of CG819 appeared to be produced in coiled or bundle-like structures in the agar milieu (Figure 2c). This appearance is probably a result of the normal conidiation process in this fungus, where phialoconidia are produced in terminal chains.

While some strains of *Metarhizium* have been reported to be able to produce submerged conidia under certain culture conditions (Fargues *et al.* 2001), the genetic control of this character and its high variability among strains is unknown. Since strain CG423 normally sporulates poorly in submerged culture, our results suggest that the genome of co-transformant CG819, probably carries an insertion of one of the marker genes in a locus that regulates this characteristic. This mutant

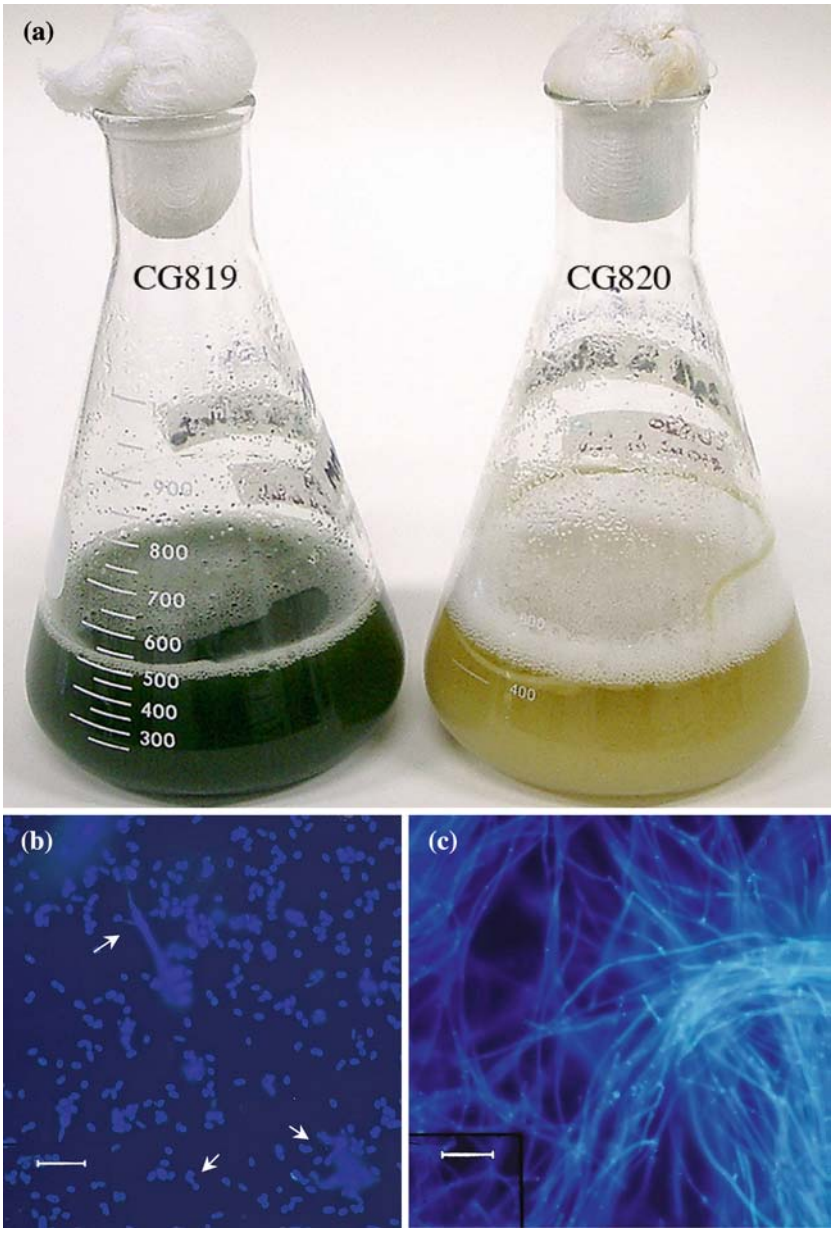

Figure 1. Submerged conidiation in a shake flask culture of *M. anisopliae* var. *acridum* insertional mutant CG819, compared with co-transformant CG820, carrying the same marker genes (Panel a). One litre Erlenmeyer flasks containing 500 ml *Aspergillus* minimal medium with Hutner's trace elements, supplemented with 2.0 g l^{-1} yeast extract, were inoculated with 1×10^6 conidia and incubated for 96 h at 28 °C with shaking at 200 rpm. Panels b and c show the general aspect of submerged cultures of CG819 and CG820 respectively, stained with the fluorescent dye, Tinopal (bar = 20 μm).

should therefore be useful in the molecular genetic analysis of submerged conidiation in *Metarhizium*. Such data may then permit the directed mutagenesis of other strains, permitting a wider assessment of submerged conidiation as an alternative method for the bulk production of infective propagules in strains normally lacking this characteristic.

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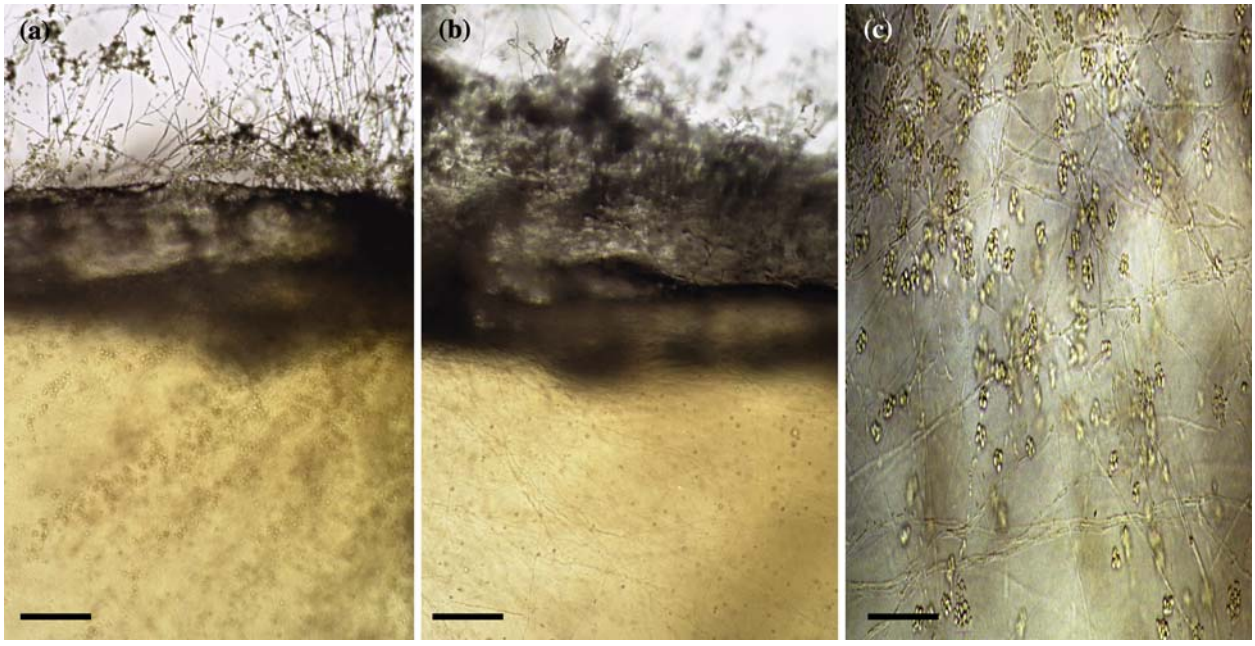

Figure 2. Slide cultures of *M. anisopliae* var. *acridum* insertional mutant, CG819 (Panels a; bar = 250 μm and c; bar = 50 μm), compared with co-transformant CG820 (Panel b; bar = 250 μm). Sections (1.5×1.5×0.5 cm) of solidified potato dextrose agar were placed on sterile microscope slides and a sterile cover slip placed over the agar. Each of the four exposed, 0.5 cm deep, faces of agar were then inoculated with conidia and incubated at 28 °C for 5 days.

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