Chapter 6

Production of Conidia by the Fungus *Metarhizium anisopliae* Using Solid-State Fermentation

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Abstract

This chapter describes the production of conidia by *Metarhizium anisopliae* using solid-state fermentation. Before production of conidia, procedures for strains conservation, reactivation, and propagation are essential in order to provide genetic stability of the strains. The strain is conserved in freeze-dried vials and then reactivated through insect inoculation. Rice is used as a substrate for the conidia production in two different bioreactors: plastic bags and tubular bioreactor. The CO_2 production in the tubular bioreactors is measured with a respirometer; this system allows calculating indirect growth parameters as lag time (t_{lag}) (25–35 h), maximum rate of CO_2 production ($rCO_{2\,max}$) (0.5–0.7 mg/gdm h), specific rate of CO_2 production (μ) (0.10–0.15 1/h), and final CO_2 production (CO_2) (100–120 mg/gdm). Conidial yield per gram of dry substrate (gdm) should be above 1×10^9 conidia/gdm after 10 days of incubation. Germination and viability of conidia obtained after 10 days of incubation should be above 80% and 75%, respectively. Bioassays using of *Tenebrio molitor* as a host insect should yield a final mortality above 80%.

Key words Entomopathogenic fungi, *Metarhizium anisopliae*, *Tenebrio molitor*, Solid-state fermentation, Biological control

1 Introduction

In the last six decades, chemical pesticides have been the most used tools in insect control or against weeds and plant diseases; however, the continuous accumulation affects the environment and the human health [1]. Alternative programs, such as those based on classical and augmentative biological control and sterile insect techniques are likely to provide effective and sustainable options for the control of native and exotic pests [2]. Once wild fungal isolates have been recovered from agricultural fields, and identified as entomopathogenic strains, feasible conservation methods are essential for long-term studies or industrial productions; additionally, those are reliable methods to preserve genetic stability of the strains [3]. Solid-state fermentation (SSF) is the preferred system

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