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IMPROVING PRODUCTION AND QUALITY OF CONIDIA BY OXIDANT STATES

Octavio Loera.
Department of Biotechnology, Universidad Autónoma Metropolitana-Iztapalapa, C.P. 09340, Mexico City, MEXICO.
Email: loera@xanum.uam.mx
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Mycoinsecticides are based on conidia from entomopathogenic fungi (**EF**), which are then applied in crop fields to control insect plagues, either as alternatives to or as enhancers of chemical insecticides (1). The main species of **EF** belong to the genera *Metarhizium, Beauveria* and *Isaria*. Conidia are mostly produced by solid cultures (**SC**) where a desirable yield is at least 10^9 conidia per gram of dry substrate. The manipulation of culture conditions determines higher levels or better productivities in **SC** (1). In order to achieve these yields some key factors are the use of stable strains and vigorous seed cultures, comprising accelerated germination, suitable colonization and resistance to different type of stresses found in common culture media, as stress promotes conidiation resulting from a cell differentiation process. For instance, fungi produce reactive oxygen species (ROS) as a result of their cellular metabolism or as a response to oxidant states. ROS are cellular signals and trigger conidiation, although beyond a critical threshold they produced oxidative stress during fungal growth (2, 3).

Fungal isolates respond in a strain dependant manner to culture conditions, then it is necessary to analyse the response of a particular strain in strategies to increase conidiation yields. The strain of *Metarhizium anisopliae* CP-Oax improved conidiation with oxidant pulses (26% O_2) during culture (4). However mycelial capacity to response to these stimuli depends on the culture time, since conidiation only improve when stimuli are applied within a narrow time interval (60 h); applying oxidant pulses outside this interval was detrimental (5). As the intensity of the stimuli has to be controlled, concentration of O_2 was varied between 16 and 40%, comparing results with atmospheric condition (21%). The conidial yield doubled in cultures subjected to O_2 at 26 and 30%. However, best productivity was observed under 30% O_2, in fact maximal yield was reached at 132 h, while under atmospheric conditions the highest yield was reached after 156 h.

Mycoinsecticides require also both proper quality traits and resistance against environmental conditions, in open fields and during storage of formulated preparations. Some quality parameters such as germination and viability of conidia relate to virulence, while some traits are associated to abiotic factors in terms of UV resistance, thermal and osmotic stress tolerance (3).

After being exposed to a specific type of stress, fungal cultures produced conidia showing resistance to other types of stresses, this is known as a cross protection phenotype (3, 6). Generally cultures under sub-lethal stress produce conidia displaying high quality parameters, although the production yields of conidia are substantially low (1). There is a great interest in finding novel approaches aimed at improving conidial quality without affecting production yields. Some culture conditions affecting conidial yields in **EF** also influence quality parameters. When *Isaria fumosorosea* was exposed to high levels of oxygen during culture (26% O_2), conidia improved germination, tolerance to osmotic stress and thermotolerance in comparison to those conidia obtained from normal conditions (21% O_2), and this was confirmed with different strains of *I. fumosorosea* (6). The physiological studies determine optimal time to applied stimuli and the level of stress tolerated by the fungi, once both parameters are defined, application of those conditions in culture increase simultaneously conidial yields and quality, as reported with *M. anisopliae* under 30% O_2 (5). Culture conditions alter metabolic responses by **EF**, the biochemical changes associated with the improvement of both conidiation levels and quality traits include the expression of genes regulating the onset of conidiation and the biosynthesis of compatible solutes protecting biomolecules from damage during exposure to a sub-lethal stress (7). There is also a response mediated by antioxidant enzymes such as catalases and superoxide dismutases, which are linked to the beginning of conidiation but also to the virulence of the conidia produced under those conditions (8).

These are some recent strategies showing how culture conditions modify production and quality of conidia by **EF**, which are currently used as alternatives in biological control.

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