SOLID CULTURES AS TOOLS FOR CELL PHYSIOLOGY RESEARCH: CASE STUDIES ON ENTOMOPATHOGENIC FUNGI

Octavio Loera. Department of Biotechnology, Universidad Autónoma Metropolitana-Iztapalapa, C.P. 09340, Mexico City, MEXICO. Email: loera@xanum.uam.mx

Key words: conidial yields and quality, oxidative stress

Entomopathogenic fungi (**EF**) belonging to different genera such as *Beauveria, Metarhizium, Isaria* and *Lecanicillium* play a main role in the biological control as alternatives to chemical pesticides. As reproductive forms in **EF**, blastospores are produced in liquid media, while conidia are mainly produced in solid cultures. Relative to blastospores, conidia are mostly used in biological control due to higher virulence and resistance to adverse environmental conditions, such as temperature, UV radiation and desiccation (1); in addition, conidial are more hydrophobic, which eases the initial interaction with insect cuticle (2).

In the production of conidia by **EF**, the most used substrates are rice, barley, oat, sorghum grains and wheat bran, which can also be mixed with a variety of co-substrates or texturizers in order to improve conidial yield or productivity (2). After optimization of conditions, and despite this diversity of substrates, the highest yields are between10^9 to 10^{10} conidia per gram of dry substrate, which is equivalent to 100 grams of conidia per kg of initial dry substrate (3).

The parameters considered in those optimization processes include the level of inoculums, associated to prediction of harvesting times since this reduce the maintenance and costs of the culture; the use of suitable texturizer have also been proposed to improve the low thermal conductivity and the micro-gradients (*e.g*. nutrients, water activity) found in solid cultures (2).

In solid cultures, a special concern has to be considered for the effect of conditions such as chemical composition, water activity, aeration rate, different types of stresses (nutritional, osmotic, thermal, oxidative), since every small change in any of these parameters could affect the final conidial yield and productivity (3,4). However, in batches of conidia, quality features such as virulence and resistance to abiotic factors found in open fields are not commonly reported in the production strategies (1, 4).

For strains of *Beauveria bassiana*, the chemical composition of the media had a differential effect on fungal growth rate and conidial yields (up to one order of magnitude), which simplifies the selection of the preferred carbon source for every strain when scaling up the process. This is even more critical since only a minor effect was observed on infectivity parameters (5). Current studies on solid cultures using inert supports, imbibed with define media, analyse the proteomic expression according to particular carbon sources or culture trait to be tested during conidia production. Similarly, on line automated monitoring systems for CO_2 and O_2 in solid cultures allow the identification of respiratory transitions associated with the onset of conidiation (6).

Aerobic organisms produce reactive oxygen species (ROS) as a result of their cellular metabolism, which signal the beginning of conidiation in filamentous fungi (7).

In solid cultures the oxidative stress can be applied by atmospheric pulses varying the oxygen concentration relative to normal conditions (21%). When a moderate oxidative stress was applied to *Beauveria bassiana* using rice as a solid substrate, the highest conidial yield was achieved under low oxygen pulses (16% O_2), with no difference between normal concentration (21%) and high oxygen pulses (26%) (3). Nonetheless, germination of the conidia produced was negatively affected when the fungus grew either with 16% or 26% O_2 pulses, even when the expression of the gene ***mpd,*** involved in the synthesis of mannitol (an antioxidant solute), was up regulated. The antioxidant response by enzymes was diminished under oxidant pulses, especially catalases, causing molecular damages with emphasis in the lipid fraction of the cells (3). The susceptibility of each strain to stand the stress has to be considered. For instance, in contrast to *B. bassiana*, conidial yield with *Metarhizium anisopliae* was 100% higher under oxidant condition (26% O_2 pulses); in fact conidia yield in relation to biomass ($Y_{C/X}$) was 2.6 times greater ($1.12x10^7$ conidia per mg of dry biomass) under 26% O_2, compared to the 21% O_2 atmosphere. It was noteworthy that production of conidia in 26% O_2 atmosphere did not affect virulence on *Tenebrio molitor*.

These are some recent and current studies showing how solid cultures take advantage of diverse techniques, including proteomics, molecular biology, respirometry and controlled stress in order to contribute on the production of high levels of conidia by **EF**, improving the productivity while maintaining the quality of every batch.

Acknowledgements

To the project CONACyT:152420-Z, UAM-Iztapalapa and Red Promep for the joint financing of these studies.

1. Miller CD, Rangel D, Braga GUL, Flint S, Kwon SI, Messias CL, Roberts DW, Anderson AJ (2004), *Can J Microbiol* 50:41–49

2. Nuñez -Gaona O, Saucedo-Castañeda G, Alatorre-Rosas R, Loera O (2010). *Braz Arch Biol Techn* 53:771–777

3. Garza-López PM, Konigsberg M, Gómez-Quiroz LE, Loera O (2012). *World J Microbiol Biotechnol*, 28, 353–359

4. Tlecutil-Beristain S, Viniegra-González G, Díaz-Godínez G, Loera O (2010) *Mycopahologia* 169:387–394

5. Rodríguez-Gómez D, Loera O, Saucedo-Castañeda G, Viniegra-González G. (2009) *World J Microbiol Biotechnol*. 25: 513–8.

6. Saucedo-Castañeda S, Trejo MR, Lonsane BK, Navarro JM, Roussos S, Dufour D, Raimbault M (1994) *Process Biochem* 29: 13-24

7. Aguirre J, Ríos-Momberg M, Hewitt D, Hansberg W (2005) *Trends Microbio*l 13(3):111–118