How to test heat, osmotic, and oxidative tolerance of Neurospora conidia and young mycelia.

Nora Plesofsky

How to test thermotolerance of conidia in liquid culture.

1. Inoculate conidiospores into 2% sucrose-Vogel's salts medium at a concentration of 1 mg/ml, and incubate with vigorous shaking at 30ºC for two hours to eliminate the protective effect of dormancy.

2. Transfer flask with conidial culture to a shaking water bath at the test temperature. In the cited article, we found that conidia exposed to 48ºC and higher for 2 hr showed sharply reduced survival.

3. To quantify survival, dilute an aliquot of spores 5,000-fold into 10% Vogel's salts. We commonly do this in series by adding 0.2 ml of spore suspension to 9.8 ml of diluent in 20x150 mm glass tubes, vortexing to mix, and adding 0.1 ml of this dilution to 9.9 ml of diluent in a second tube.

4. Add 50 µl of the final spore dilution to 10 cm diameter petri plates, containing Vogel's medium with 1% sorbose, 0.05% glucose, and 0.05% fructose. We commonly make a 10-fold concentration stock of the sorbose, glucose and fructose, which is autoclaved and stored at 4ºC. This is added to the water-Vogel's salts-agar mix, after the latter is autoclaved, before pouring the media. Spread spore dilution over the solid medium with glass spreader (made from long Pasteur pipette shaped by heating); the spreader is sterilized by ethanol and flaming.

5. The plates are incubated at room temperature (26ºC), and the colonies derived from surviving conidia are counted after 2-4 days or when visible. Comparison should be made with a control treatment, in which conidia are diluted and plated after only the initial 30ºC incubation.

6. One can also test the protective effect of a 1 hr heat shock at 45ºC, which is the optimum temperature for heat shock protein synthesis, against subsequent exposure to a lethal temperature, e.g., 50ºC for 2 hr.

How to test osmotic tolerance of conidia in solid medium.

1. Incubate conidia in 0.05% glucose-Vogel's liquid shake culture at 30ºC for 2 hr, as described above; initial sugar concentration makes no difference for this short interval.

2. Dilute conidia 5,000-fold, as above, and plate onto sorbose-glucose-fructose-Vogel's medium, containing 1 M NaCl; incubate the plates at room temperature (26ºC). Under these conditions, we found that wild type survived 20%, and the osmotic-2 mutant strain did not survive. No survival was seen for wild type at 2 M NaCl. Comparison was made with cells plated on medium lacking NaCl addition.

How to test tolerance of conidia to oxidative stress.

1. Incubate conidia in 0.05% glucose-Vogel's medium 30ºC for 2 hr, as described above.

2. Dilute conidia 5,000-fold and plate onto sorbose-glucose-fructose-Vogel's medium, containing different concentrations of hydrogen peroxide, added after plate medium is autoclaved. We found that wild type cells survived ~6% in 0.6 mM H_2O_2, ~1% in 0.7 mM H_2O_2, and 0% in 1 mM H2O2, as compared with cells plated on medium lacking H_2O_2.

How to test stress tolerance of young mycelial cells in liquid culture by determining their rate of death.

1. Germinate conidia in glucose-Vogel's medium in liquid shake culture for 5 hr.

2. Add the physical or chemical stressor, and at predetermined time points (e.g., every hour) remove 100 μl of the cell culture, using a trimmed pipette tip for larger bore opening.

3. Mix culture aliquot with 100 μl of 0.4% of the vital stain trypan blue (dissolved in 1X Vogel's salts and filtered with a 0.45 micron filter), and shake the mixture well for 15-30 min.

4. Add ~8 ul of mix to microscope slide. Count the germ tubes from dead or dying fungi that have retained the blue dye, dividing this number by the total number of germ tubes in your field of vision. It is optimal to count a total of 300 or more germinating conidia.

5. We have determined the kinetics for 8 hr or until there is greater than 90% death.

Reference:

Plesofsky-Vig, N. and Brambl R., 1985, J. Bacteriol. 162:1083-1091