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Mutant enrichment by filtration concentration: a variation for the selection of temperature-conditional heterocaryons

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Abstract

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The inclusion of sorbose into a medium used for filtration concentration greatly facilitates the separation of growing mycelia from non-growing conidia, and also permits the recovery of individual isolates without the intervening step of plating samples of the primary culture on an agar-solidified medium containing sorbose. In addition, because growth is restricted with sorbose, the primary culture need only be filtered at intervals more compatible with the diurnal rythum of a civilized investigator, i.e., = 12 hours. The following is a general procedure using such a medium for the selection of heterocaryon derivatives with dominant temperature-conditional alterations.

Conidia from a two-week old minimal medium culture of a stable, nutritionally-balanced heterocaryon are suspended in sterile water, filtered through spun glass wool to remove large mycelial clumps, and exposed to a mutagen (only ultraviolet light has been used). Treated conidia ore suspended in 250 ml of Fries minimal salts containing 6% (w/v) sorbose, 0.5% (w/v) glucose and 0.5 (w/v) fructose in a 500 ml Erlenmeyer flask at a concentration of co. 1×10^5 survivors on minimal medium/ml. The culture is agitated at 160 rpm (gyrotary) at a temperature determined to be non-permissive to remove the growing, non-mutant conidia by filtration concentration (37° C for the selection of heat-sensitive derivatives and 15° C for the selection of cold-sensitive derivatives). Every 12 hours, the culture is filtered through a combination of gauze-type cheesecloth and spun glass wool such that the "porosity" of the filter material is decreased at successive filtrations. When no growth is apparent for a period of 24 hours, the culture is shifted to a temperature that will permit the growth of the desired derivatives (23° C for recovery of heat-sensitives and 27° C for cold-sensitives) and agitation continued at 120 rpm. The culture is incubated at the permissive temperature until conidia that con grow after the temperature shift form 1-2 mm mycelial colonies. The culture fluid is then decanted from the flask and the colonies washed with sterile water and individually cultured under appropriate conditions to confirm their temperature conditional phenotype. Because only minimal medium is used throughout filtration concentration, a high proportion of there isolates are temperature-conditional heterocaryons (usually between 1/10 and 1/20).

The above procedure has been used to detect mutations in genes whose functions are required for nuclear multiplication or for the transfer of information from gene to cytoplasm. • • School of Life Sciences, University of Nebraska, Lincoln and the Department of Physiological Chemistry, University of Wisconsin, Madison: