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PRODUCTION OF PROTOPLASTS IN AN OSMOTIC MUTANT OF *NEUROSPORA CRASSA* WITHOUT ADDED ENZYME

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ABSTRACT

HAMILTON, JAMES G. (Tulane University School of Medicine, New Orleans, La.), AND JANET CALVET. Production of protoplasts in an osmotic mutant of *Neurospora crassa* without added enzyme. J. Bacteriol. **88**:1084-1086. 1964.—An osmotic mutant of *Neurospora crassa* was grown in minimal medium containing increasing concentrations of sorbose. Protoplasts were produced in the higher concentrations by inhibition of cell-wall synthesis. Similar molar concentrations of fructose, glucose, and sucrose also produced protoplasts, suggesting a relationship between inhibition of cell-wall synthesis and osmotic pressure. Increased sugar concentration led to decreased growth.

The production of protoplasts in various microorganisms has simplified the study of their biochemistry, morphology, and genetics. In the ascomycete *Neurospora crassa*, protoplasts have been produced in wild-type and four chemical mutants by use of the enzyme preparation, snail gut juice (Bachmann and Bonner, 1959). Emerson and Emerson (1958) produced protoplasts in a *Neurospora* osmotic mutant with the use of a commercial hemicellulase preparation. In both of these studies, the fungus was grown to the hyphal stage, and the cell wall was incompletely digested by enzymes. In this study, another approach was assumed; i.e., protoplasts were produced by inhibition of cell-wall formation.

de Terra and Tatum (1961), in a study of colonial morphology in *Neurospora*, showed that hyphae became bulgy when grown in 4% sorbose medium. The hyphae resembled protoplasts surrounded by a weakened cell wall. In testing several *N. crassa* mutants on sorbose media in this laboratory, it was found that the osmotic mutant M16 produced small colonies composed almost entirely of protoplasts. Perkins (1959) described this mutant as unable to grow in a medium to which 4% sodium chloride has been added. The purpose of this paper is to describe the production of protoplasts in osmotic mutant M16 without adding enzyme and to discuss the significance of the results.

MATERIALS AND METHODS

Osmotic mutant M16 (obtained from the Fungal Genetics Stock Center, no. 812) was used for the entire study. Vogel's (*unpublished*) meddium and modifications of it were used throughout the study. The composition of Vogel's medium is as follows (in grams per liter): sucrose, 20.0; Na_3 citrate·$5.5H_2O$, 3.0; KH_2PO_4 (anhydrous), 5.0; NH_4NO_3 (anhydrous), 2.0; $MgSO_4 \cdot 7H_2O$, 2.0×10^{-1}; $CaCl_2 \cdot 2H_2O$, 1.0×10^{-1}; citric acid·$1H_2O$, 5.0×10^{-3}; $ZnSO_4 \cdot 7H_2O$, 5.0×10^{-3}; $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, 1.0×10^{-3}; $CuSO_4 \cdot 5H_2O$, 2.5×10^{-4}; $MnSO_4 \cdot 1H_2O$, 5.0×10^{-5}; H_3BO_3 (anhydrous), 5.0×10^{-5}; $Na_2MoO_4 \cdot 2H_2O$, 5.0×10^{-5}; and biotin, 5.0×10^{-6}.

Cultures were grown in 100 ml of minimal medium containing 5, 10, 15, and 20% filter-sterilized sorbose (Mann Research Laboratory, New York, N.Y.). Cultures were inoculated with a loop from a 24-hr slant and were grown at room temperature for 2 to 3 weeks. Microscopic examination of cultures was performed by use of wet mounts. Lysis of protoplasts occurred when 1 or 2 drops of water were added under the cover slip.

To test various sugars as carbon sources and osmotic pressure stabilizers, additional cultures were grown as above in minimal medium (without the usual 2% sucrose) plus 5, 10, 15, and 20% concentrations of sucrose, fructose, glucose, and sorbose.

RESULTS

In minimal medium, hyphae of M16 were microscopically identical to the wild type (Fig.

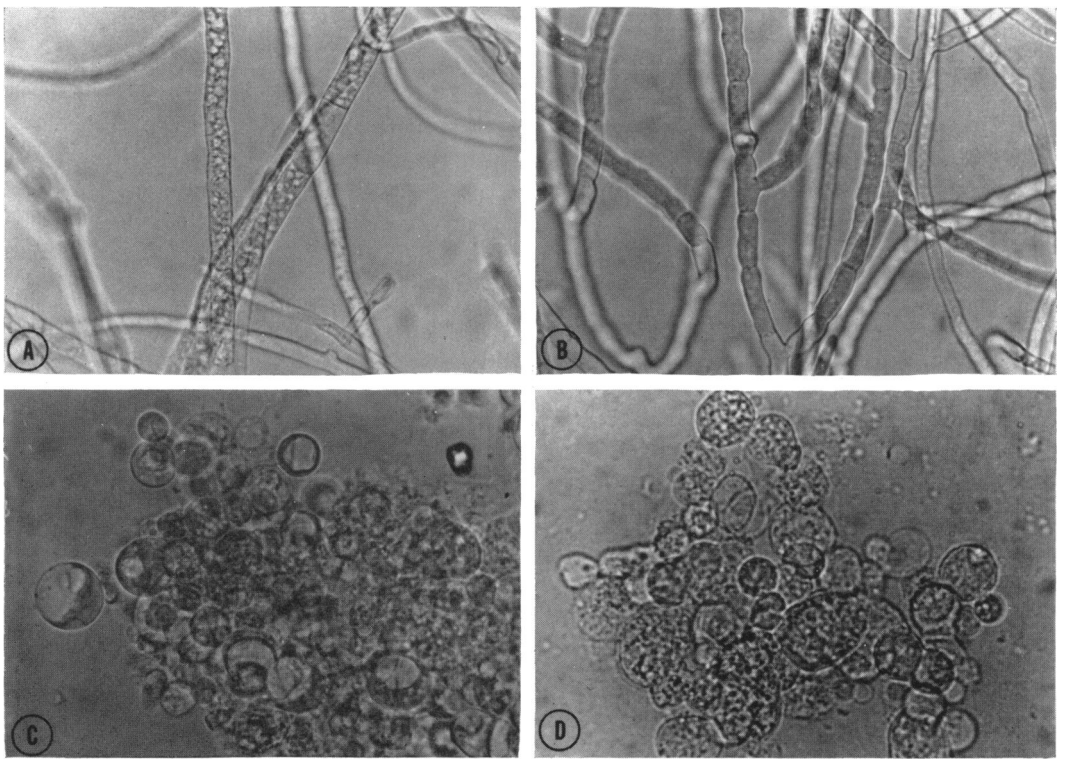

FIG. 1. *Osmotic mutant M16 grown in Vogel's medium with various concentrations of sorbose. Magnification, 570 ×. A, minimal medium; B, minimal medium plus 5% sorbose; C and D, minimal medium plus 10% sorbose.*

1A). Minimal medium plus 5% sorbose caused a bulging of hyphae similar to that produced by sorbose on the wild type (de Terra and Tatum, 1961; Fig. 1B). Minimal medium plus 10% sorbose decreased cell-wall formation, producing spheroplasts, protoplasts, and occasional bulgy hyphal fragments (Fig. 1C and D). Minimal medium plus 15 and 20% sorbose produced structures similar to those in Fig. 1C and D, except a greater proportion was osmotically sensitive.

Growth did not occur in any concentration of sorbose until 2% sucrose was provided as a carbon source. Glucose, sucrose, and fructose served as both carbon sources and osmotic stabilizers. Results with the latter three sugars were similar to those with sorbose plus 2% sucrose when considered on a molar basis; i.e., 20% sucrose produced about the same results as did 10% glucose, fructose, or sorbose plus 2% sucrose. Growth inhibition occurred with increasing sugar concentrations, and appeared to be approximately proportional to osmotic pressure. As cultures became older, some individual protoplast colonies began to form hyphae. This occurred with all sugars and did not seem to be related to osmotic pressure.

To test for viability and sensitivity of protoplasts, agar plates containing minimal medium plus 10% sorbose were inoculated with protoplasts from a 15% sorbose liquid culture. Under the microscope, a single protoplast was observed multiplying into two, four, and then a cluster of protoplasts. Transfer of the colony to a slide and the addition of 1 or 2 drops of water under the cover slip showed most to be osmotically sensitive.

DISCUSSION

Inhibition of growth and cell-wall formation of the osmotic mutant M16 seems to be related to osmotic pressure, since similar inhibition occurs in equimolar concentrations of glucose, fructose,

sucrose, and sorbose (with 2% sucrose). Although the biochemical defect in this mutant is unknown, it appears likely that it involves the cell membrane, since the membrane is thought to be the regulator of materials passing into and out of the cell. Although it seems likely that protoplast formation is a result of cell-wall synthesis inhibition by extracellular loss of necessary substrates or enzymes, an alternative explanation would be the extracellular movement and activation of an autolytic enzyme.

The spherical bodies produced in 20% fructose, glucose, or sorbose-sucrose meet the criteria for *Neurospora* protoplasts as set forth by Bachmann and Bonner (1959); i.e., they are viable and osmotically sensitive. In lower sugar concentrations, the spherical bodies do not meet these criteria as well, since the varying amounts of cell wall present decrease the osmotic sensitivity.

ACKNOWLEDGMENT

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