Fungal Genetics Reports

Volume 13 Article 4

The care and feeding of slime

V. W. Woodward

C. K. Woodward

Recommended Citation

Woodward, V. W., and C.K. Woodward (1968) "The care and feeding of slime," *Fungal Genetics Reports*: Vol. 13, Article 4. https://doi.org/10.4148/1941-4765.1937

The care and feeding of slime

Abstract

The care and feeding of slime

Woodward, V. W. and C. K. Woodward.

The care and feeding of slime.

The Fungal Genetics Stock Center maintains, in heterocaryons, five isolates or forms of slime (for stack see fz; sg; os-1), referred to as sl in this paper. We have worked only with the stocks numbered FGSC# 327 and 1118. The most complete description, to date, of sl is presented by Emerson (1963 Genetica 34: 162) and in two abstracts, available through the Stock Center.

sl can be separated from the mycelial components of the heterocaryons using the "filtration" technique (Woodward et al. 1954 Proc. Natl. Acad. Sci. U.S. 40: 192), after which it can be cultured on agar slants or in liquid medium. Regular Vogel's medium supplemented with 2% soluble starch, 0.75% yeast extract and 0.75% nutrient broth, is good for both agar and liquid media; the mutant grows well when supplemented with 2 % sucrose, but during the first 48 hours of incubation it produces hyphlets which later "shed" their wall-like material before assuming the slime appearance. Preliminary analyses of this wall-like material indicate that it lacks amino sugar and galactose polymers, i.e., it probably is a glucose polymer of ß-1,3 glucan.

When grown on agar slants the mutants grow as blobs resembling bacterial colonies, but with little or no internal compartmentalization. The entire "colony" is surrounded by a membrane. When the membrane is broken the cytoplasm flows out onto the agar forming small sphericles from 10-90µ in diameter, resembling in every way the sphericles seen in growth in liquid shake cultures. We have seen no evidence for the tendency of these sphericles to "divide"; rather, we conclude that the agitation by shaking is responsible for the increase of sphericles. The best way to maintain the cultures for long periods of time is in heterocaryons; however, for shorter durations the mutants can be maintained on agar slants at room temperature if they are transferred every 7-14 days. In liquid culture, the mutants begin logarithmic growth after 12-18 hours, reach stationary phase after 48-72 hours, and die after 96 hours. However, after 72 hours most of the sphericles have ruptured, leaving either membrane fragments or hollow spheres.

To grow sl in bulk, the trick is to find an optimum liquid volume-agitation ratio. We have been unsuccessful in our attempts to grow sl in large carboys under forced aeration, but we have not exhausted all of the environmental combinations. However, we have been successful in growing relatively large quantities in liter Erlenmeyer flasks on a rotating shaker. Seed cultures are maintained by transfer every 48 hours into 50 ml medium in 250 ml Erlenmeyer flasks. These flasks are incubated at 30°C on a reciprocal shaker (90 strokes per minutes). From 48-hour-old cultures approximately 1×10^7 cells (0.5 cc medium) ore used to inoculate 200 ml liquid medium in liter Erlenmeyer flasks, which in turn are incubated at 30°C on a rotary shaker (100 rpm). After 48 hours the cells are collected by centrifugation (500 x g for 5 minutes). The supernatant is removed by suction after which the cells are taken up in 25 times their volume of Tris-sucrose-EDTA buffer (0.05 M Tris, 0.5 M sucrose, and 4×10^{-3} M EDTA, at pH 8.5) and are then combined with an equal volume of 3 mm diameter glass beads. The mixture is rotated at 40 rpm far one hour in a jar mill at 4°C; most of the cells ore broken by this procedure.

Our interest in sl developed following great difficulty in isolating membrane-enzyme complexes, viz., aspartate transcarbamylase, from wild type Neurospora. After demonstrating the existence of such a complex in sl, it became obvious that sl could be used to isolate enzyme aggregates of various sorts, and also to isolate plasma membrane. We have succeeded in isolating plasma membrane and in comparing the "structural" protein with mitochrondrial structural protein and have found them to be identical as judged by amino acid composition and polyacrylamide gel electrophoresis patterns. We are also using sl to study membrane formation.

Following the recent Neurospora Information Conference at Asilomar, California, it became evident that sl might prove useful to many of the investigations discussed there. As a result, we have been asked to present these details for maintaining the mutants and to present in a general way the direction of our own research with sl. (PHS Grant No. GM-15137-01, and Univ. of Minn. Graduate School.) - - - Deportment of Genetics, University of Minnesota, St. Paul, Minnesota 55101.