How to work with *slime* and other cell wall-deficient strains.
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Background

At the first Neurospora conference in 1961, Sterling Emerson showed living cultures of his newly discovered *slime* strain, in which absence of the cell wall enabled nuclei, nucleoli, and mitochondria to be clearly visualized under phase contrast microscopy. A full description followed (Emerson 1963). Genetic analysis revealed a complex, multigenic basis. Methods were developed for preserving the fragile *slime* cultures and maintaining them in sheltered condition in heterokaryons. Crosses were made in an effort to obtain progeny having the same or improved characteristics (Emerson 1963, Nelson *et al*. 1975).

The original strain and its derivatives provided a unique resource for a variety of studies, most notably of the plasma membrane and associated entities. (For a summary of uses, and for references, see Scarborough 1985, 1988; Perkins *et al*. 2001.) The potential of *slime* for cell biology and for cytological observations has probably not yet been fully exploited.

One essential genetic component of Emerson's *slime* strain is the osmotic-sensitive gene *os-1* (allele B135). A separate line of research with cell wall-deficient strains has employed a temperature-sensitive allele, *os-1* ^{NM233t} (Selitrennikoff *et al.* 1981)

Procedure

Slime:

The following is adapted from Nelson *et al.* (1975):

"*Slime* is stored as a component of the heterokaryon FGSC 327 (*fz; sg; arg-1cr-1 al-1 os-1* (B135) A + *al-2 nic-1 lys-3 os-1* (E11200) A) at –20°C. It can be resolved from the heterokaryon by filtration concentration in a liquid medium (medium A) containing 1× Vogel's salt solution, 10% sorbose, 2% sucrose, 2 mM arginine. Stock cultures can be maintained in liquid medium B, which contains 1× Vogel's salt solution,, 7.5% sorbitol, 1.5% sucrose, 2 mM arginine) or in medium B solidified with agar. [Antibiotics were added to both media to guard against growth of contaminating bacteria. Chloramphenicol would supposedly now be used.] Cells from stocks of *slime* that were recently resolved from heterokaryon FGSC 327 can form heterokaryons with cells from filamentous strains of like mating type and *het* genotype, or with cells of opposite mating type that carry the *tol* gene (e.g., FGSC 1949, *tol pan-1* a), [or presumably with *helper-1* and similar helper strains that have since become available]. Continuous cultivation of *slime* cells by serial transfer twice weekly to shake liquid medium results in the loss of both heterokaryon-forming capacity and male fertility within 2 months(o Selitrennikoff *et al*. 1970).

Growth of newly resolved stocks of slime in shaken liquid medium is erratic, and as Emerson noted, the wall-less *slime* cells (spheroplasts) and wall-like cell debris tend to form large irregular agregates. By filtration through spun glass wool at each successive transfer to fresh medium stocks can be obtained which grow as a homogeneous suspension of spheroplasts; such trained stocks have characteristic and reproducible logarithmic growth rate constants (cf. Woodward and Woodward 1968). Occasionally, in older liquid stock cultures, the spheroplasts become filled with vacuoles and, microscopically, resemble soap bubbles with little protoplasm. Other properties also become altered. Such stocks should be discarded.

Spheroplasts from cultures of slime which are growing exponentially in liquid medium B can be plated on agar-solidified medium B with 100% recovery: Cell suspensions are gently spread out on the surface of the solid medium and the excess liquid is allowed to slowly diffuse into the agar. when a suspension of spheroplasts is agitated for 2 minutes with a Vortex mixer, the number of potential colony-forming units doubles; when the suspension is agitated for longer intervals, the number of colony-forming units declines. Single colonies of *slime* can be transferred to fresh solid medium with a sterile toothpick; this technique can be used to streak out individual cells of a colony or to prepare a grid of 50-100 colonies per 9 cm plate. After 2 days growth on solid medium at 33°C, groups of colonies can be accurately replica plated with velveteen-covered blocks, as is routinely done with bacteria or yeast."

A variant with improved properties has been described (FGSC 4761) (Scarborough 1985).

Nelson *et al.* (1975) constructed a mixed-mating type heterokaryon (FGSC 2713) and used it in crosses with filamentous strains to recover *slime* and *slime*-like progeny.

Stocks can be maintained conveniently as forced heterokaryons that are phenotypically wild type (FGSC 1119, 327, 2713). Once formed, the heterokaryons are transferred to agar slants of standard Vogel's minimal medium, allowed to conidiate, and preserved using standard methods for conidiating cultures.. Alternatively, individual *slime* strains may be stored at −70°C (Creighton and Trevithick 1973, modified by Sebo and Schmit 1980). Liquid cultures are grown to a density of 2×10^7 cells/ml and 2 ml samples are frozen at −80°C. Selitrennikoff (1978) recovered *slime* from petri dish and slant cultures frozen *in situ* and stored at −70°C.

The following is from Wilson 1986:

"Strains to be stored over liquid nitrogen are suspended in 10% DMSO. Approximately 0.2 ml of the suspension is pipetted into glass ampules as used for lyophilization. These are flame sealed and secured to aluminum canes, two to a cane. Each ampule and each cane is marked with the FGSC number of the strain. These are frozen just as the samples were for lyophilization. Five ampules are usually prepared, with one to be used as a control. Immediately after freezing, the control ampule is dropped into a beaker of 35°C water. The contents are withdrawn with a pasteur pipette and dispensed onto an appropriate medium to check viability after freezing and thawing. If the control is viable, it is assumed the other four samples frozen at the same time are too.

Some additional steps are taken when freezing slime (*fz;sg; os-1*, FGSC 1118 and 4761). The freezing rate is slowed. Following a suggestion made by G.A. Scarborough, ampules are loaded into a balsa wood tube plugged with cotton. The whole tube is suspended in liquid vapor for 15 minutes, by which time the samples have frozen. On thawing, the ampule contents are withdrawn and dispensed into 3 ml of liquid minimal medium in the belief that washing (by dilution) the DMSO is beneficial."

os-1^{NM233t}

As described by Selitrennikoff *et al.* (1979), growth and morphology of *os-1^{NM233tt}* are normal at 25°C and mutant at temperatures above 34°C. Protoplasts are formed when macroconidia (10^4 cells/ml) are incubated with shaking at 37°C in Vogel's liquid medium N containing 1.5% sucrose, 10% sorbose, and 200 μg/ml polyoxin B. Initial cultures are a mixture of protoplasts and wall-bounded cells. Enrichment for protoplasts is accomplished by daily filtration into fresh medium through sterile glass wool. After 30 days, cells are transferred to Vogel's liquid medium N plus 1.5% sucrose and 7.5% sorbitol (no polyoxin) and incubated for an additional 7 days at 37°C (transferred daily). Cultures are then a homogeneous

population of protoplasts that are stable, failing to regenerate cell wall at 37°C even in absence of polyoxin. Most protoplasts are nucleated and their structure is very similar to that described for *slime*.

Morphologically normal cell walls are regenerated when cells are transferred to medium containing 7.5% sorbitol and incubated at 25°C.

Note on availability of polyoxin: The polyoxins are competitive inhibitors of chitin synthase. They are unavailable from commercial sources, or prohibitively expensive. However, The Fungal Genetics Stock Center has several kilograms of a crude mixture of polyoxins donated by R. M. Metzenberg, who obtained it from Japan. (Contact FGSC for details.) A procedure for partial purification to give ~50% polyoxin B is given by Selitrennikoff (1984).

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