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Toward the domestication of slime.

Unless otherwise indicated, the term "slime" refers to the Neurospora crassa strain FGSC #326 [fz (no #); sg (no #); arg-1 (B369), cr-1 (B123), our (34508), os-1 (B135)A], originally isolated by Sterling Emerson. As compared with filamentous strains, slime is particularly suitable for cell fractionation, ond as such, could be a powerful tool in understanding the molecular biology of N. crassa. With this in mind, we have developed simple genetic and microbiological techniques for handling slime.

Natural history. Slime is stored as a component of the heterocoryon FGSC #327 [fz;sg;arg-1, cr-1, aur, os-2A [illegible], lys-3, os-2A] at -20° C. Slime can be resolved from this heterocoryon by filtration concentration in a liquid medium (medium A). ining Ix Vogel's salt solution, 10% (w/vl sorbose, 2% (w/v) sucrose, 2 mM arginine, 50 µg/ml chloramphenicol, 50 µg/ml streptomycin, and 100 units/ml potassium penicillin G (Emerson 1963 Neurospora Newsl. 4: 19-20). Stock cultures can be maintained in liquid medium B (Ix Vogel's salt solution, 7.5% (w/v) sorbitol, 1.5% (w/v) sucrose, 2 mM arginine. and antibiotics as used in medium A) medium B solidified with agar. Cells from stocks of slime recently resolved from heterocaryon FGSC X327 can form heterocaryons with cells from filamentous strains of like mating type (A) and with cells from strain FGSC #1949 [tol (N83), pan-1 (5531)a]; these "young" slime cells con also fertilize the protoperithecia formed by filamentous strains of opposite mating type (a). Continuous cultivation of slime cells by serial transfer twice weekly to shaken liquid medium results in the loss of both heterocoryon-forming capacity and male fertility within two months.

Growth of newly resolved stocks of slime in shaken liquid medium is erratic, and or Emerson noted (1963 Genetica 34: 162), the wall-less slime cells (spheroplasts) and wall-like cell debris tend to form large irregular aggregates. By filtration through spun glass wool at each successive transfer to fresh medium, stocks con be obtained which grow as a homogeneous suspension of spheroplasts; such trained stocks hove characteristic and reproducible logarithmic growth rote constants (cf. Woodword, V.W. and C. K. Woodward 1968 Neurosp. Newsl. 13: 18).

Occasionally, in older liquid stock cultures, the spheroplasts become filled with vacuoles ond, microscopically, resemble soap bubbler with little protoplasm. When this occurs, other properties characteristic of younger stocks also become altered. Such stocks should be discorded.

Microbiological methods. Spheroplasts from cultures of slime which ore growing exponentially in liquid medium B can be plated on agar-solidified medium B with 100% recovery: cell suspensions ore 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 rpheroplortr is agitated For two minutes with a Vortex Genie, the number of potential colony-Forming units doubler; when the suspension is agitated for longer intervals, the number of colony-Forming units declines. Single colonies of slime con be transferred to Fresh solid medium with a sterile toothpick; this technique con be used to streak out individual cells of a colony or to prepare a "grid" of 50-100 colonys per 9 cm plate. After two days growth on solid medium at 33° C, groups of colonies con be accurately replica plated with velveteen-covered blocks, as is routinely done with bacteria or yeast.

Standard methods for treating Neurospora conidia with ultra-violet light give high rates of killing of slime spheroplasts. Preliminary results indicate that this treatment con be used to induce new mutations directly in slime.

Construction of the compatible mixed mating type heterocoryon H2 A/o [fz (no #); sg (no #); arg-1 (B369), cr-1 (B123), our (345081), os-1 (B135) A + tol (N83), pan-1 (5531) a]. In a heterocaryon, slime doer not lose mole fertility; a mixed mating type heterocoryon offers the Further advantage that only the slime component will act as the male parent in appropriate crosser. The compatible mixed mating type heterocoryon H2 A/a is prepared as Follow. Slime (FGSC #326) is grown on solid medium B For one week at 33° C. Slime cells are then suspended in liquid medium B without the orginine supplement and mixed with dry conidio from strain FGSC #1949 [tol, pan-1 (5531) a]. The mixture is placed in the center of a 9 cm petri plate containing solid medium B without orginine. The tol mutation suppresses A/o heterocoryon incompatibility (Newmeyer 1968 Genetics 60: 207); we Find that the probability of heterocaryon formotion is increased by incubation at a high temperature (33° C). Once formed, the heterocaryon is transferred to agar slants of standard Vogel's minimal medium; the cultures are incubated at 33° C, allowed to Form conidio, and then stored at -20° C.

We have deposited a stock of strain HZ A/a with the FGSC.

Crossing slime ond slime heterocaryons with filamentous strains and the recovery of slime progeny. In crosses involving slime, true slime segregants hove never been recovered (Emerson 1963 Genetica 34: 162). Therefore, a filtration enrichment procedure is used in preference to total isolation for recovery of slime progeny. The non-slime parent strain is induced to Form protoperithecia on standard synthetic crossing medium. The protoperithecia are Fertilized with spheroplasts From a young slime stock or with conidio from strain H2 A/a. Ascospores are collected in water ond washed twice by gravity sedimentation through 6% (w/v) potassium sodium tartrate to remove any associated conidio or hyphae. Without prior heat shock, the washed ascospores ore suspended at 1-5 x 10^4 spores/ml in 150 ml of medium C (Ix Vogel's salt solution, 10% (w/v) sorbose, 2% (w/v) sucrose, 0.02% (w/v) Difco agar, 4 X 10^{-3} M furfuryl alcohol, antibiotics as used in the other media, ond appropriate nutritional supplements). The 150 ml culture is incubated in a 500 ml Erlenmeyer flask in a gyro-rotatory shaker (130 rpm) at 30° C. Each day, the culture is Filtered to remove filamentous progeny. During the first two weeks, the porosity size of the Filter is gradually reduced using various combinations of gauze-type cheesecloth and spun gloss wool as filter materials. After ca. 2-3 weeks, 10 ml of the culture, which should be "cloudy" with speroplasts, are diluted in 40 ml of medium B (with appropriate supplements) in a 125 ml Erlenmeyer flask; this secondary culture is incubated in a gyro-rotatory shaker at 30° C and filtered every other day through four layers of spun glass wool. After ca. two weeks, sampler of the culture are agitated with a Vortex Genie (to increase the proportion of homocaryotic spheroplasts), plated out on solid medium B and incubated at 33° C for three days. As noted by Emerson (ibid.), colonies of "slime-like" strains have a superior crust ond subsurface hyphoe. On the other hond, colonies of true slime strains hove a wet-appearing surface and do not Form hyphoe. Individual colonies of the latter type ore transferred to 13 X 100 mm cul-

ture tubes containing 0.5 ml of liquid medium B, dispersed with a Vortex Genie, and incubated at 33° C. The volume of each culture is slowly increased to 3 ml by daily additions of fresh medium. Strains of the desired genotype(r) ore stored at -70° C using the method of Creighton and Trevithick (1973 Neurosp. Newsl. 20: 32) and/or at -20° C in a heterocaryon.

To dote we hove recovered true slime strains with markers on LG-IL (cys-11), LG-IR (nuc-1) or LG-VR (pho-2).

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