CALIFORNIA INSTITUTE OF TECHNOLOGY

Division of Biology

Notes on culture of the SLIME variant of *Neurospora crassa*

(1207-1A)UV-1 (A arg-1 cr aur os fz sg) -- the original slime variant, obtained following U.V. irradiation of Perkins' 1207-1A (arg-1 cr aur os), requires arginine (and biotin). FGSC # 1121

E b413-6A (A os fz sg) -- isolated following 4 generations of outcrossing to wild types, no nutritional requirement (other than biotin). FGSC #1118

Culture of slime stocks. I use Vogel's N medium with 2% sucrose, biotin (at least 5 micrograms/liter), supplemented with arginine (25 mg/100 ml) for (1207-1A) UV-1. Media are solidified with 1.5% Difco agar.

Growth on solid medium does not penetrate into the agar, consists of slime flows at margins of colonies, with clumps of spherical cells occurring in older, thicker parts (at least some of these spherical cells have cell walls).

Growth in liquid culture consists of clumps of spherical cells and short, thick, contorted hyphae, both usually with delicate cell walls. Cells are easily ruptured, either mechanically or by osmotic shock. Growth is fairly similar regardless of the osmotic concentration of the medium.

Viability of slime cultures is poor. Agar cultures must be transferred frequently. In liquid cultures of high osmotic concentration (say 10% sucrose), viable cells can be recovered after several months. Cultures do not survive refrigeration.

Heterocaryons. Because of the poor viability of slime cultures, stocks are maintained in heterocaryons with hyphal phenotypes. The arginine requiring (1207-1A)UV-1 is kept opposite Perkins' 1413-2A (al-2 lys-3 nic-1 os) which requires lysine and niacin. The heterocaryon grows on minimal medium, E b413-6A, FGSC #1118 which has no growth requirement, is kept opposite 1207-1A (arg-1 cr aur os), the arginine requiring parent of the original slime. This heterocaryon also grows on minimal medium.

FGSC # 326

Recovery of slime from heterocaryons. Heterocaryotic hyphae are inoculated into liquid medium of high osmotic concentration (I use 10% sorbose, which is inert, 2% sucrose, standard salts), supplemented to select for the slime component. For (1207-1A)UV-1/1413-2A, supplement with 50 mg/100 ml l-arginine to restrict growth of the lysine-requiring component; for b413-6A/1207-1A with 50 mg/100 ml l-lysine to restrict growth of the arginine-requiring component. Growth in both instances should consist of slow-growing, contorted hyphae and of spherical cells which are budded off from the hyphae and continue growth by budding or fission. Every few days, filter through sterile glass wool to remove the hyphal growth. When only spheres and short, stubby hyphoids are produced in two days after filtering, plate on an agar medium containing 5% sorbose, 2% sucrose (plating on minimal medium often ruptures cells by osmotic shock). Persistent slime colonies are transferred on small blocks of the agar on which they are growing to tubes of minimal agar (arginine supplemented for the arginine-requiring slime). Some isolates may still be heterocaryotic, and produce hyphal outgrowths on the minimal medium.

FGSC # 327

FGSC #1119

The genetic basis of the slime phenotype is complex, and still somewhat obscure -- see Emerson, S., Genetica (1963) 34: 162-182.