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Siderophore transport mutants (sit) in Neurospora crassa.

During the last several years we have isolated and worked with a number of sideraphore transport mutants (sit) in Neurospora crassa. The work is unfinished and nothing has been published about these mutants, except for their listing in the "Compendium" (Perkins, et al., 1982 Microbiol. Rev. 46: 426). Since one of us (GWC) is no longer working in this field, we have decided to make the mutants available to anyone interested. This note introduces the mutants, their background, isolation and general characteristics. They have been deposited in the Stock Center (FGSC 4211 through 4231) in the current stock list.

Background: The parent strain is the triple mutant, arg-5,ota,aga (FGSC 2744), which was obtained from R.H. Davis. This mutant is blocked in all known pathways of ornithine synthesis and produces so little ornithine (Charlang and Ng, 1982 Neurospora Newsl. 29: 15) that it becomes siderophore-dependent when grown in medium with glycerol as carbon source. This observation formed the basis for use in "the hunt for" siderophore transport mutants.

Isolation: To reduce siderophores pools in conidia, the triple mutant was grown without ornithine prior to the hunt. UV-treatment of conidia was followed by filtration enrichment in glycerol medium supplemented with ferricrocin (FC) (Vogel's N-free salts; glycerol and asparagine). All media contained putrescine and arginine (recrystallized) which were filter-sterilized and added to the autoclaved medium. All liquid media also contained Tween 80 [four drops (56 mg) per 100 ml]. FC was added to the autoclaved medium at 10 to 20 µg per 100 ml. Ungerminated conidia were plated on sorbose medium supplemented with ornithine. One thousand isolates from these plates were tested for growth in liquid glycerol medium (same medium as above, except Winkelmann's salts (Winkelmann and Zahner 1973 Arch. Mikrobiol. 88: 49) instead of Vogel's, with trace elements added (but not Fe or citrate) supplemented with either ornithine or FC. Those 120 isolates that grew on ornithine but not on FC were tested for ^{3}HFC uptake. The medium used in the uptake was the liquid glycerol medium (Winkelmann's salts) used in the growth tests of the original isolates. Conidia were germinated for 3 hours at 30°C before measuring uptake.

Eleven isolates were found to be uptake deficient. To check specificity of the uptake defect they were tested for ^{14}C-phenylalanine transport. Six of the 11 isolates appeared to have a general transport defect as they were also deficient in phenylalanine uptake. They have been set aside and not analyzed any further. (They are not in the Stock Center but GWC still has silica gels, if anyone would like to have them.) The remaining five isolates were normal as far as phenylalanine transport is concerned, and they were designated as sit mutants.

Crosses: The five sit mutants were crossed to 74a in order to remove the parental marker genes. Progeny were first analyzed in low water activity (a_w) medium with or without ferricrocin. This test was based on our earlier finding that, in low a_w media (a_w ~0.93), conidia lose that part of their ferricrocin pool which is required for germination (Horowitz et al., 1976 J. Bacteriol. 127: 135). Germination will occur, however, if the medium is supplemented with the siderophore. Uptake mutants should show little or no response to added ferricrocin. The medium was the same as that used for uptake studies, except that sucrose was used as C-source and NaCl was added to lower the a_w to .966. At this level wild type strain 74a still shows a response to added ferricrocin. Any a_w lower is severely inhibitory to the triple mutant. Any progeny looking like uptake mutants were then tested for "HFC uptake and analyzed for presence of parental genes.

Removal of the parental genes proved to be difficult in most cases because not only are arg-5, ota and aga in separate linkage groups, but two of them interfered with the progeny testing; arg-5 reduces ferricrocin uptake, and aga increases sensitivy to low a_w. We did succeed in removing the parental genes from the mutants in all but one case; sit-5 still carries ota.

Before these crosses were completed others were set up between sit mutants to test for allelism. The five mutations appear to be nonallelic. (Some of the double mutants obtained at that time have been deposited in the Stock Center. Many still carry the arg-5 genotype and may be of limited usefulness in siderophore transport studies.)

The mutations have not been mapped.

General Description: The sit mutants grow well on minimal medium. sit-1 germinates slowly. sit-2 has a deep orange color, and its conidia tend to remain connected in twos or threes. sit and sit-4 are very poor protoperithecial parents.

None of the mutants is totally unable to transport FC or coprogen. There is some passive FC and coprogen uptake in Neurospora, and this may be what we are observing in these mutants. sit-1 and sit appear to be binding mutants. Their ability to bind FC to the cell surface (in presence of NaN_3 which inhibits active transport)

is much lower than that of wild type strain 74A. The other sit mutants have normal binding.

Current Work: Shortly before the demise of our group at Caltech, we had begun a biochemical search for the siderophore receptor using sit-l, sit-2 and a double mutant (sit-l , sit-2). This work was initiated by Lennart Adler who is continuing the studies at Gothenburg University, Sweden.

One of us (NPW) plans to continue the genetic analysis of the mutants to establish the location of the five sit loci in the Neurospora linkage groups.

We thank N.H. Horowitz for support, guidance and encouragement. (Supported by Public Health Service Grant AI-15739.) Division of Biology, California Institute of Technology, Pasadena, California 91125 and Department of Botany, Howard University, Washington, DC 20059.
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