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New *or-2* and *or-5* alleles.

While selecting macroconidiation-defective mutants of *N. crassa*, we isolated three strains whose morphology was similar to that of an osmotic mutant. We now present data confirming that two are allelic to *os-2* (ALS10) and the third is allelic to *or-5* (NM2160).

74-OR8-1a macroconidia were treated with ethyl methane sulfonate (25 μ l/ml) for two hours at 35°C and surviving macroconidia (ca. 10% of input) were incubated for five days at 35°C on plates containing 1% sorbose, 0.1% sucrose solid medium. All colonies which were morphologically distinguishable from wild type were transferred to tubes containing 1 ml Vogel's N solid medium and incubated for three days at 35°C. Among ca. 3500 colonies transferred, we isolated three strains (CPS 80, CPS 84 and CPS 93) which were morphologically similar to *os-1* (B135). Each of these three strains was backcrossed to wild type (Oak Ridge) four times and a reisolate used for the experiments described below.

The operational definition of an osmotic mutant is that its growth is inhibited by the addition of 4% NaCl to the medium (Perkins 1959 Genetics 44: 1185). In the presence of high salt concentrations, the growth of the mutant strains was inhibited (Table 1) providing further evidence that CPS 80, CPS 84 and CPS 93 are osmotic mutants.

From genetic analysis we conclude that CPS 80 and CPS 93 are alleles of *os-2* and CPS 84 is on an allele of *os-5*. Those crosses which demonstrate allelism are presented in Table 2. Complementation results (Table 3) exactly confirm the allelic relationships determined by the recombination data.

STRAIN	NaCl CONCENTRATION			
	0	0.4~	0.8M	1.2M
74-OR8-1a	88	71	37	16
<i>os-5</i> (NM 2160)	89	59	10	0
<i>os-2</i> (ALS 10)	90	43	4	0
CPS 80	71	45	17	0
CPS 84	73	55	7	0
CPS 93	68	50	3	0

Table 1. Effect of NaCl concentration on growth of osmotic and wild-type strains. Numbers indicate dry wt. in mg. after 5 days growth at 25°C in 25 ml Vogel's N medium supplemented with 0.23% Na-Acetate, 0.01% Tween 80 and the NaCl concentration shown (modified from Mays 1969 Genetics 63: 781).

STRAIN	1	2	3	4	5
1. <i>os-2</i> (ALS10)	•	•	•	•	•
2. CPS 93		-	-	*	*
3. CPS 80			-	*	*
4. CPS 84				-	-
5. <i>os-5</i> (NM2160)					-

The new *os-2* alleles and the new *os-5* allele have been deposited in the Fungal Genetics Stock Center for use by interested investigators. (This work was supported in part by an NSF predoctoral traineeship to CPS). - - - Department of Zoology, University of California, Los Angeles, Los Angeles, California 90024.

CROSS	PROGENY		VIABILITY ^a
	wild type	osmotic	
CPS 80 x CPS 93	0	126	63
<i>os-2</i> (ALS 10) x CPS 80	0	133	67
<i>os-5</i> (NM 2160) x CPS 84	0	118	67

Table 2. Recombination of osmotic mutants.

Crosses were made on corn meal agar. Individual ascospores were transferred to solid Vogel's N medium and progeny scored on basis of morphology after 5 days growth at 25°C. a = % germination.

Table 3. Complementation of osmotic mutants.

Heterokaryosis was established using *ad-2* (Y175M256) and *arg-10* (8137) as forcing markers under conditions non-selective for the osmotic mutations (Vogel's N solid minimal medium). Heterokaryons were transferred to the same medium + 4% NaCl and growth scored after 3 days at 25°C. - = no growth; • = wild-type growth.