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

While selecting macroconidiotion-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 ore allelic to os-2 (ALS10) and the third is allelic to *or-5* (NM216o).

74-OR8-1a macroconidia were treated with ethyl methane sulfonate (25 µl/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 distinguish&e from wild type were transferred to tubes containing 1 ml Vogel's N solid medium and incubated for three doys at 35°C. Among ca. 3500 colonies transferred, we isolated three strains (CPS80, CPS84 and CPS93) which were morphologically similar to os-1 (B135). Each of these three strains was bockcrossed to wild type (Oak Ridge) four times and a reisolate used for the experiments described below.

The operational definition of on 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 CPS80, CPS84 and CPS93 are osmotic mutants.

From genetic analysis we conclude that CPS80 and CPS93 ore alleles of os-2 and CPS84 is on allele of os-5. Those crosses which demonstrate allelism ore 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
os-5(NM216o)	89	59	10	0
os-2(ALS10)	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 ond 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.

CROSS	PROGENY wild type	PROGENY osmotic	VIABILITY[a]
CPS 80 x CPS 93	0	126	63
os-2(ALS10) x CPS 80	0	133	67
os-5(NM 2160) x CPS 84	0	118	67

Table 2. Recombination of osmotic mutants.
Crosses were mode on corn meal agar. Individual ascospores were transferred to solid Vogel's N medium and progeny scored on basis of morphology after 5 doys growth at 25°C. a = % germination.

STRAIN	1	2	3	4	5
1. os-2(ALS10)	•	•	•	•	•
2. CPS 93		-	-	*	*
3. CPS 80			-	*	*
4. CPS 84				-	-
5. os-5(NM216o)					-

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.

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