Fungal Genetics Reports

Volume 21 Article 8

A simple classroom complementation experiment

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Recommended Citation
Selitrennikoff, C. P., and M. Bailey (1974) "A simple classroom complementation experiment," *Fungal Genetics Reports*: Vol. 21, Article 8. https://doi.org/10.4148/1941-4765.1801

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Abstract

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Selitrennikoff, C. P. and M. Bailey. A simple classroom complementation experiment.

The utility of Neurospora in an undergraduate genetics laboratory course for demonstrating basic genetic principles has been previously described (Neurospora in teaching 1966 NN#10: 15). A reliable, visual method for demonstrating intergenic complementation is presented here. This procedure exploits the observation that csp-1 and csp-2 strains produce conjoined conidio so that when agar slant cultures are inverted and sharply tapped no freed conidio are observed falling - the "tap test" (Selitrennikoff and Nelson 1973 NN#20: 34). However, when either wild-type cultures or forced heterokaryons of csp-1/csp-2 are topped, they release an easily observed copious cloud of freed conidio.

The one allele of csp-1 (UCLA37) and the three alleles of csp-2 (FS591, FS590, UCLA 101) were each crossed to nic-2 (43002) and nit-3 (Y31881) and heterokoryon compatable double mutant cultures isolated (Selitrennikoff; in preparation). These compatable strains can be used in a classical "cis-trans" test requiring only the top test to detect the presence or absence of complementation. To illustrate, we present an experiment which was performed by the Fall 1973 Genetics Laboratory students at California State University at Fullerton. Each group of 3 students was given agar slant cultures (Vogel's medium N + 1.5% sucrose + 50 µg/ml nicotinamide) of the following strains (each tube was labeled with only the allele number and the appropriate auxotrophic locus designation) and 20 sterile slants of Vogel's N + 1.5% sucrose minimal medium.

1. nic-2 A
2. nic-3 A
3. csp-1 (UCLA37), nit-2 A
4. csp-1 (UCLA37); nic-3 A
5. csp-2 (FS590); oic-2 A
6. csp-2 (FS590), nic-3 A
7. csp-2 (FS591); nic-2 A
8. csp-2 (FS591), nit-3 A
9. csp-2 (UCLA 101); nic-2 A
10. csp-2 (UCLA 101); nic-3 A.

Table 1. Heterokaryotic cultures were formed by co-inoculation of aerial hyphae onto minimal medium. After 7 days' growth at 25°C, tubes were scored by the tap test.
+ = free conidio
- = conjoined conidia.

STRAIN	nic-2	UCLA37 nic-2	FS590 nic-2	FS591 nic-2	UCLA10 nic-2
nic-3	+	+	+	+	+
UCLA37 nic-3		-	+	+	+
FS590 nic-3			-	-	-
FS591 nic-3				-	-
UCLA101 nic-3					-

The students were asked to design their own experimental protocol in order to obtain data which would allow them to answer these questions: 1) Is each morphological mutant gene recessive to its wild-type allele? 2) How many loci do the 4 mutant genes i.e., UCLA37, FS591, FS590 and UCLA 101, represent? All groups independently performed the heterokaryon analysis with the results indicated in Table 1 and concluded that FS590, FS591 and UCLA 101 were functional alleles, UCLA37 was a separate locus, and all were recessive. The above IO strains are available from the Fungal Genetics Stock Center.

This work was supported in port by on NSF predoctoral traineeship to CPS. - - - Deportment of Biological Sciences, California State University, Fullerton, CA 92634.