Complementation Between Histidine Requiring Mutants of *Saccharomyces cerevisiae*

Introduction

Complementation experiments test for function. If two mutations separately produce a phenotype but when combined into a diploid/heterozygote state correct the defects of the other and recapitulate the wild type phenotype, then they are said to complement each other. Two mutations are noncomplementary if the mutant phenotype persists when they are combined. The organism used in this experiment, *Saccharomyces cerevisiae* which can form diploid as well as haploid cells, allows for the use of complementation analysis. Complementation tests provide information about the genetic location of a particular mutation. Complementing mutations are on different genes and noncomplementing mutations are within the same gene. It is also possible to distinguish between intergenic complementation (as described above) and intragenic complementation where two mutations are found within the same gene. Generally mutations within the same gene would not revert back to wild type; however, if they are two *different* mutations within the same gene then the wild type phenotype can be observed. Intragenic complementation is characterized by slower growth and reduced activity or heat stability of the affected enzyme (Jeyabalan 57). This experiment used complementation assays to find out which gene in the yeast histidine biosynthesis pathway a particular unknown mutation is located and also to observe intergenic versus intragenic complementation.

Materials and Methods

See the *Genetics 306 Lab Manual* for details of the procedure (Jeyabalan 60-63). Refer to **Table 1** for known A and α strain mutations and note that A and α have secondary auxotrophic mutations in *different* genes. If complementation occurred between mutant strains then growth

would be present at the diploid mating junctions on the minimal medium indicating that the wild type histidine synthesizing pathway was restored.

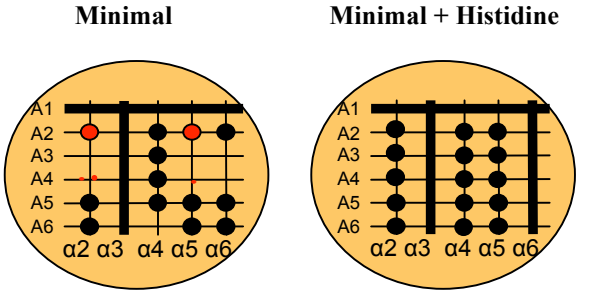
Table 1: Known A and α strain mutations

Strain Number	1	2	3	4	5	6
A: Known Mutations	$His7^-$ Trp^-	$His4C2^-$ $Trp1^-$	$His4C3^-$ Ino^-	$His4A^-B^-C^-$ $Leu2^-$	$His5^-$ Leu^-	$His1^-$ $Leu2^-$
α: Unknown Mutations	Ade^-	Ade^-	Ade^-	Met^-	Ade^-	Ade^-

Results

After one and two days of incubation, growth was evident at all mating junctions on the minimal medium except α2/A2, α2/A3, α2/A4; α4/A6; α5/A2, α5/A3, α5/A4; and α6/A3, α6/A4. On the fifth day growth appeared at α2/A2 and α5/A2. Confluent lines of growth were present along A1, α3, and α6. A few small colonies were present at the α2/A4 and α5/A4 mating intersection after five days of incubation. See **Figure 1** for a picture depiction of the growth on the minimal medium and minimal medium plus histidine plate. Growth was present at all of the mating junctions on the minimal plus histidine plate. Confluent growth was found along A1 and α3 lines.

Figure 1: Results of A and α matings on minimal and minimal plus hisitidine medium plates



Red colonies were not present until day five
Black lines represent confluent growth

Discussion

All of the matings should grow on the minimal plus histidine control plate because the different secondary auxotrophic requirements for each strain A and α would complement the other in the diploid state. This plate was used to ensure matings were efficient and that the second auxotrophic mutations were present. The results show growth at all mating junctions indicating that the

correct matings occurred; however, there was confluent growth along A1, α3, and α6. This could happen if the strain lost its second auxotrophic requirement so these results indicate that A1, α3, and α6 are no longer trp^-, ade^-, and ade^-, respectively.

The growth patterns on minimal medium were used to generate **Table 2.** Growth at a mating junction on the minimal plate implies that the wild type histidine synthesizing pathway was restored due to complementation between the two histidine mutations in each strain. Complementation between the two strain's secondary auxotrophic mutations must have also occurred if growth is present on the minimal plate.

Table 2: **Table of complementation results after one, two, and five days of incubation and the genetic locus of α strain mutations**

Strain	α2	α3	α4	α5	α6
A1	+	+	+	+	+
A2	Day 1&2 (-) Day 5 (+)	+	+	Day 1&2 (-) Day 5 (+)	- / (+)*
A3	-	+	+	-	-
A4	-	+	+	-	-
A5	+	- / (+)*	+	+	+
A6	+	+	-	+	+
Mutation Location	**$His4C^-$** (not $His4C_2^-$)	**$His5^-$**	**$His1^-$**	**$His4C^-$** (not $His4C_2$)	**$His4C^-$**

Plus and minus signs designate growth or lack of growth, respectively, at mating junctions
*Expected results are in black and observed results are in red

The genetic locus of the histidine mutation in each α strain was determined by first identifying the mating(s) that did not complement, that is, where no cells grew at the junction on minimal medium. The known mutation in the A strain at that junction would indicate the gene that is mutated in the α strain because mutations at the same genetic locus do not complement each other. Using this logic, the α4 strain did not complement with A6 indicating that it has a mutation in the his1 gene, **Table 2**.

After one and two days of incubation the expected non-complementing diploids for the α2, α5, and α6 strains were A2, A3, and A4. The common mutation in these A strains is his4C

which specifies that the mutation in α2, α5, and α6 is in the his4C gene. However, since his4C_2 in A2 and his4C_3 in A3 are closely linked it is not possible to determine the exact genetic location within the his4C gene the α mutations are in after only two days of incubation. After five days of incubation, α2 and α5 complemented with A2. Such slow growth is characteristic of intragenic complementation and indicates that the mutated gene in α2 and α5 is not his4C_2 but could be his4C_3 or another mutation within the his4C gene. Since α6 did not complement after five days (expected results) the mutation could be within hisC_2 or $_3$ or another his4C gene. Notably, the observed results show that α6 *did* complement A2. A possible explanation is that this mating junction became contaminated with a complementary strain during the plating procedure.

The expected results show that the α3 strain should not complement with A5 indicating that it has a mutation in his5, but the observed results show growth at this junction. **Figure 1** shows that confluent growth was along the α3 line in this minimal medium which could only happen if this strain lost both of its auxotrophic mutations, his and ade, and reverted to wild type. The A1 strain also shows a full line of growth on this plate indicating that it lost both histidine and tryptophan mutations.

The genetic event responsible for the few small colonies at the intersections of the noncomplementing strains α2/A4 and α5/A4 could be mutational reversion or suppression. The histidine and other auxotrophic mutations could revert back to their wild type forms, or a second site could be mutated which also reverts back to the wild type phenotype.

Summary

This experiment used complementation analysis to map the genetic locus of five unknown histidine mutations, **Table 2**. Intergenic and intragenic complementation was observed.

The observed results varied from the expected results by showing confluent growth on minimal and minimal plus histidine medium which confer information about which mutations have been lost in the strains. Finally, mutational reversion was noted after five days of growth at the junction of several noncomplementing strains.

References

Jeyabalan, S. Fall 2005. *MCDB 306 Genetics Laboratory Manual*. University of Michigan. 57-63.