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Thermal Tolerance Phenotype in cdc13-1 exo1□□ Heterozygous Diploids of *S. Cerevisiae* is a Dominant Trait

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ABSTRACT:

Background: Telomeric DNA is found at the end of eukaryotic chromosomes, where they play a role in protecting the chromosome and the integrity of the genome of the organism through the activity of telomerase. *Saccharomyces cerevisiae* exists in two genotypes: haploid and diploid. Temperature sensitive point mutation on the cdc13 gene of each genotype and deletion of exo1 gene (*cdc13-1*Exo1□ mutants) give rise to mutant survivors at enhanced temperatures. The mode of inheritance of the thermal tolerance allele in the heterozygous diploid genotype is not known.

Materials and Methods: We constructed diploids by mating temperature sensitive haploid strains of opposite mating type *cdc13-1* exo1: LEU with temperature resistant strains of *cdc13-1* exo1::HIS. The crosses were 1296x3182 (D) and 2561x3181 (C). Using a sterile stick, smear of one haploid strain was made on each YEPD plates labelled C2, C8, C10, D4, D10, and D113. A smear of another opposite mating type was made on the previous strain. They were mixed and allowed to mate for six hours, before culturing on media lacking Luecine and Histidine (–L and –H) to purify and confirm that they are diploids. After confirmation, a loop full aliquot of the diploids were streaked on sterile media lacking leucine and histidine (-L, -H) and on YEPD and cultured at 37^0C to check thermal tolerance and number of viable colonies from each diploid crosses in (cfu).

Result: The heterozygous diploid D thrived at the enhanced temperature of 37^0C and there is a significant difference in the yield of viable colonies by the D diploids when compared to the yield of the C diploids with P-value of 0.05.

Conclusion: The growth of diploid D10 as shown in plate 3.1 establishes that, temperature resistant allele inherited by cdc13-1 exo1□□□heterozygous diploids is a dominant phenotype, and its mode of inheritance is dominant as the heterozygous diploid thrived at the enhanced temperature of 37^0C.

KEYWORDS: Temperature, haploid, Diploid, Mutation, Survivors, cdc13-1 Mutants, Allele

1. INTRODUCTION

1.1 Yeast Biology

Two types of yeasts exist, budding and fission yeast. They are ubiquitous and grow on many substances and high-sugar content substrates [1, 2]. They grow at various temperatures regimes, ranging from about 15^0C to 30^0C, with the optimum temperature range been 23^0C to about 24^0C. At higher temperature range, the yeasts enzymes becomes denatured, and they die. When yeasts are nutritionally stressed, for example by deprivation of either a carbon or a nitrogen source, diploid yeast cells sporulate. Under the microscope, yeast appears circular, with characteristic bud. Most yeast reproduce by budding [3, 4]. *Schizosaccharomyces pombe*, however, elongates as cylindrical cells until it divides by binary fission. Yeasts are very popular and are useful 'model' for genetic research because the ascospores they produce in each ascus are the products of meiosis [5].

Some yeasts species e.g., *Saccharomyces cerevisiae* can exist in two states: having one genome i.e. haploid state or two genome i.e. diploid state. Haploid cells are known to occur in two different mating types: **a** or **α** [6, 7]. The type is determined by the expression of a gene at an active mating type locus (*MAT*), and each mating type responds to a pheromone produced by the opposite mating type, as an invitation for mating. Diploid cells however do not mate, nor produce pheromone, but can undergo meiotic division to produce four haploid mating cells, while haploid cells do not undergo meiotic division nor sporulate [8]. If an **α** cell undergoes gene conversion, and replaces the *MAT***α** allele

with the *MAT***a** allele by using the silenced copy of a *MAT***a** allele present at the *HMR* (Hidden MAT Right) locus it will undergo a switch to become an **a** cell.

1.2 Cdc13-1exo1□□Temperature Resistant Mtutants Clones of *Saccharomyces Cerevisiae*

cdc13-1exo1□□temperature resistant mutants are clones of *Saccharomyces cerevisiae* with a temperature sensitive point mutation at the cdc13 gene and a deleted exo1 gene. The deletion of exo1 gene confers on the mutants, temperature resistant capabilities, which enables them to survive enhanced temperatures and as such are called surviviors. Cell cycle arrest is known to occur in *cdc13* temperature sensitive mutants of S*accharomyces cerevisiae* at the G2 phase when grown at a restrictive temperature [9, 10], due to DNA degradation which activates the *RAD9* checkpoint. According to [11], *cdc13-1 rad9* cells dividing at this restrictive temperature, contain single-stranded DNA, corresponding to telomeric and telomere-proximal DNA sequences and the temperature range to which they survive is between 23^0C to 28^0C [11, 12]. However, survivors no longer undergo cell cycle arrests at G2 phase at the restrictive temperature but instead undergo a nuclear division, exits mitosis and enter a subsequent division cycle and continue to grow, due to recombinational telomere elongation (RTE) [13, 14].

1.3 Inheritance of Temperature Tolerance Allele by Offspring of Diploids of *Saccharomyces cerevisiae*

Offspring inherit genetic traits known as allele from their parents [15, 16]. Point mutation in a gene could lead to inheritable genetic disorder [17, 18, 19]. Diploid species are known to inherit randomly, a copy of the allele from each of their parent [15, 20]. The mode of inheritance of temperature resistant allele in homozygous diploids specie and their phenotype could be predicted [21, 22], while the mode of inheritance of temperature resistance allele in heterozygous diploids specie and their phenotype remains a subject of a probability. Therefore, the mode of inheritance of temperature resistant allele (thermal tolerance phenotype) in cdc13-1 exo1□□□heterozygous diploids is not known. This study, however, was carried out to elucidate, the mode of inheritance of temperature resistance allele in cdc13-1 exo1□□□heterozygous diploids.

2. MATERIALS AND METHODS

2.1 Generation of Study Organisms

Young culture at the exponential phase of growth used for this study was sourced from the National Centre for Disease Control, Lagos Nigeria. Subcultures were made from the stock material stored at -80^0C by streaking loop full aliquot four strains of cdc 13-1 *EXO*1□ (DLY 3181, 3182, 1296 and 2561) *Saccharomyces cerevisiae* onto separate sterile YEPD media plates. The plates were properly labelled and cultured at 23^0C for 3 days.

2.2 Generation of Survivors at 36^0C

2 ml aliquot of sterile water was dispensed into 4 sterile labelled bijou bottles. Using a sterile wireloop, a loop full from a discrete colony was picked from the plate containing each strain and was mixed in each labelled bijou bottle that contained the sterile water, using a vortex mixer. The optical density (OD) for each milky suspension was measured. 0.9 ml aliquot of sterile water was also dispensed into 4 sets of 5sterile labelled bijou bottles. Each of the 4 sets of bottles was meant for each strain of *cdc13-1* mutants. The bottles were labelled ranging from 10^{-1} to10^{-5} , indicating ratios of dilution.

10-fold serial dilutions were made from each suspension into the labelled bijou bottles by measuring 100 µl aliquot from each concentrated suspension into 10^{-1} bottle, and mixed on a vortex mixer. 100 µl aliquot from the 10^{-1} bottle was also measured into 10^{-2} and mixed. This was repeated to the last dilution. The same procedure was used to dilute concentrated suspensions of DLY mutants 3182, 1296, 3181 and 2561. Each dilution bottle was mixed properly using a vortex mixer. A sterile labelled YEPD plate was divided into two equal parts, and 50 µl aliquot was measured from each bottle and spread onto each part of the plate using a sterile spreader. The concentrated, 10^{-1}, 10^{-2} and the 10^{-3} dilutions were cultured at 36^0C to generate survivors, while 25 µl aliquot was decanted from 10^{-4} and 10^{-5} dilutions and spread onto sterile labelled YEPD media plates and were cultured at 23^0C for generating and counting the numbers of viable cells in (CFU).

2.3 Generation of Heterozygous Diploids and investigation of Thermal Tolerance Phonetype

Heterozygous diploids were constructed by mating temperature sensitive and temperature resistant haploid parents. Using a stick, a smear of a temperature sensitive haploid strain was made on YEPD plate labelled C2, C8, C10, D4, D10, and D13, and a smear of another corresponding temperature resistant strain was made on the previous strain. They were allowed to mate for six hours, before culturing. We mate DLY 1296 with DLY 3182 (1296 x 3182 = D) and DLY 2561 with DLY 3181 (2561 x 3181 = C). The presumptive diploids were selected at 23^0C on media lacking leucine and histidine (-L, -H). Upon selection, they were cultured on media lacking leucine and histidine (-L, -H) and on YEPD for three days at 37^0C, the plates were divided into three parts, with the parents streaked on the top left and right, while the diploid offspring was streaked at the bottom this was to check thermal tolerance. Numbers of viable colonies from each diploid crosses were also enumerated using colony counter.

2.4 Statistical Analysis

Obtained data were statistically analyzed and the significance in difference of mean were obtained by Duncan's Multiple Range (DMR) test using SPSS20.0 software for windows SPSS, 2011.

3. RESULTS

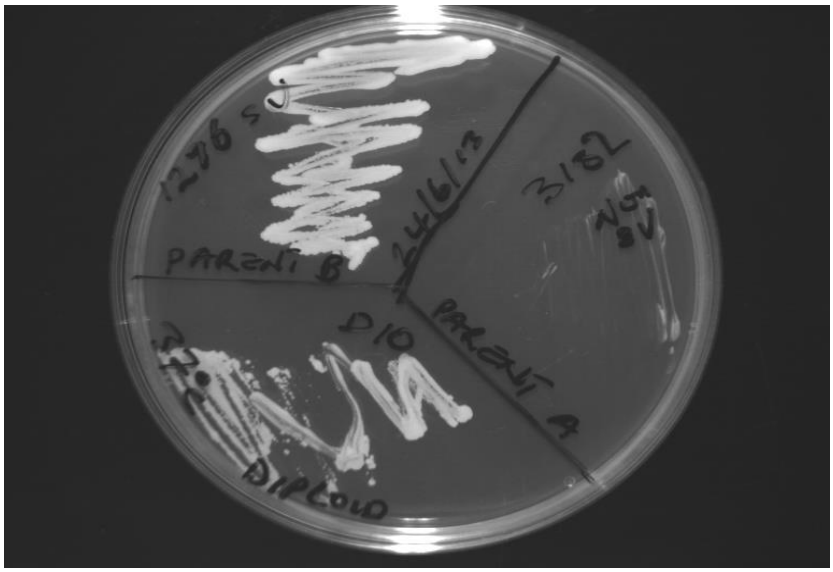

Plate 3.1: Parent A, Parent B and their heterozygous Diploid offspring

Top left: A temperature resistant strain DLY 1296 (parent B); Top right: A temperature sensitive strain 3182 (parent A); Bottom: Heterozygous diploid (D10). Note: parent B which has a temperature resistant allele, could be seen thriving at the enhanced temperature of 37^0C, parent A, which has a temperature sensitive allele could not grow at the enhanced temperature of 37^0C while their offspring D10; a heterozygous diploid which inherited a copy of temperature resistance allele from parent B and a copy of temperature sensitive allele from parent A is thriving at the enhanced temperature.

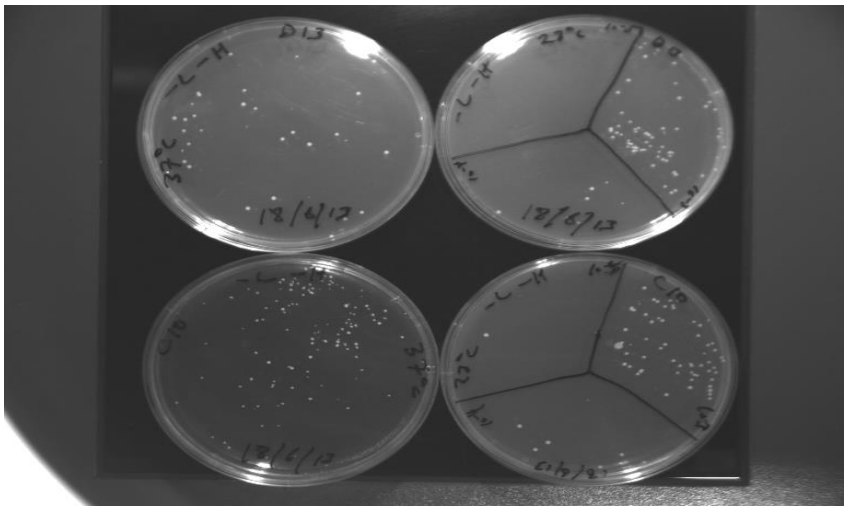

Plate 3.2: Diploids and their viable colonies in (cfu) after 3 days of growth at 37^0C, the D diploids has more viable colonies than the C diploids.

Table 3.1: Mean and Standard Deviation of Viable Colonies of the Heterozygous Diploids [(C) 2561x3181] in (cfu)

Heterozygous Diploids (C)	Mean(cfu)
C2	35±0.4
C8	38±0.2
C10	30±0.2

± = SD

Table 3.2. Mean and Standard Deviation of Viable Colonies of the Heterozygous Diploids [(D) 1296x3182] in (cfu)

Heterozygous Diploids (D)	Mean(cfu)
D4	105±0.3
D10	98±0.4
D13	101±0.2

± = SD

4. DISCUSSION

Offspring inherit discrete traits from their parents [23], which are called allele [24] in a mode that could be Mendelian [17] or non-Mendelian [19]. Point mutation in the locus of a gene could lead to inheritable genetic disorder [17, 25, 19]. Diploid species are known to inherit randomly, a copy of the allele from each of their parent [15], during segregation of traits and could be homozygous, having two same copies of allele of a gene, or heterozygous, having two different copies of allele of a gene. However, one allele in heterozygous diploids could be dominant, and the phenotype expressed in the offspring, or recessive, with the phenotype receded in the offspring [25, 26].

Co-dominance exists, though, where no allele has a complete dominance in the phenotype of the offspring [15]. The dominant allele determines the phenotype of the progeny [18, 27]. The mode of inheritance of temperature resistant allele in homozygous diploids specie and their phenotype could be predicted [18, 22]. However, the mode of inheritance of temperature resistance allele in heterozygous diploids specie and their phenotype remains a subject of a probability.

Therefore, the mode of inheritance of temperature resistant allele (thermal tolerance phenotype) in cdc13-1 exo1□□□heterozygous diploids is not known. In this study, heterozygous diploids were constructed by mating temperature sensitive and temperature resistant haploid parents. Upon selection, the diploids were cultured on media lacking leucine and histidine (-L, -H) and on YEPD at 37^0C to check thermal tolerance (see plate 3.1). At the top left of the plate is parent B; which is a temperature resistant strain DLY 1296, at the top right of the plate is parent A, a temperature sensitive strain 3182, while at the bottom is the constructed heterozygous diploid labelled (D10).

The parent B which have duplex copy of the temperature resistant allele, could be seen thriving at the enhanced temperature of 37^0C (see plate 3.1, Top left), parent A, which has a temperature sensitive allele failed to grow at the enhanced temperature of 37^0C (see plate 3.1, Top right) while their offspring D10; a heterozygous diploid which inherited a copy of temperature resistance allele from parent B and a copy of temperature sensitive allele from parent A is thriving at the enhanced temperature (see plate 3.1, Bottom), indicating that the temperature resistance allele it inherited from parent B is a dominant trait, as it manifested in its phonetic appearance. There is a significant difference in the yield of viable colonies by the D diploids which was generated by crossing DLY 1296 with DLY 3182 (1296 x 3182) when compared to the yield of the C diploids which was generated by crossing DLY 2561 with DLY 3181 (2561 x 3181) with P-value of 0.05 (see tables 3.1 and 3.2)

CONCLUSION

cdc13-1exo1□□temperature resistant mutants are clones of *Saccharomyces cerevisiae* have a temperature sensitive point mutation at the cdc13, the deletion of exo1 gene through genome editing, confers on the mutants, temperature resistant capabilities, which enables them to survive enhanced temperatures. Offspring inherit this trait from their parents**.** This study, however, has confirmed that temperature resistance in cdc13-1exo1□□temperature resistant mutants is an adaptive trait, as it only enables the organism to tolerate enhanced temperature [28, 29], it also established that in cdc13-1 exo1□□□heterozygous diploids; temperature resistant is a dominant trait.

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