MITOTIC GENE CONVERESION AND REVERSION IN SACCHAROMYCES CEREVISIAE (D7) BY ASTIBAN

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التحول الجينى الميتوزى والارتداد فى خميرة السكاروميس سرفسيا د٧ بواسطة الاستيبان

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ملخص البحث

تباين التأثير المميت للاستيبان على خلايا سلالة الخميرة د٧ من السكاروميس بر يقسيا فى الجرعات المختلفة ولم يظهر التأثير الطفرى للاستيبان فى التجربة الأولى حيث لم يتحصل على مرتدات بينما أمكن الحصول على المرتدات فى التجربة الثانية فى حالة أربع جرعات هى ٢ر٠ ، ٢ر٠ ، ٤ر٠ ، ٢٠ر٠ ميكروليتر من الاستيبان لكل ١ر٥ ملليلتر ، أما بالنسبة للتحول الجينى فقد ظهر فى كلا التجربتين ٠ هذه النتائج تبين أن للاستيبان تأثير طفرى أو ربما تأثير سرطانى على الخلايا المعاملة وذلك على الأقل فى بعض الجرعات ٠

ABSTRACT

The antibilharzian drug Astiban had different lethality effects on the diploid strain D7 of the yeast; Saccharomyces cerevisiae for the different doses used. Mitotic gene conversion and reversion were detected as a result of Astiban treatment in some doses. These results indicate that Astiban is mutagenic or probably carcinogenic at least in some doses.

INTRODUCTION

The widespread occurrence of mitotic recombination after treatment with mutagenes and the strong correlation between the convertogenic and the mutagenic activity of chemicals, that have been reported by many authors led to the consideraiton of mitotic recombination as an important consequence of genetic damage inflicted on the cellular genetic material (Siebert et al., 1970; Zimmermann, 1971 and Murthy, 1979).

Many assays had been developed for testing, mutagenicity, carcinogenicity and teratogenicity. They include mammals and short term tests. Yeast is a very appropriate and convenient organism for mutagenicity studies. It is an eukaryotic organism with a short generation time. It can be easily manipulated by microbial techniques, and a statiscally large number of yeast cells can be used in the investigation, thus providing detailed analysis of mutational events. In addition, yeast forms a bridge between prokaryotic bacteria and eukaryotic higher organisms for testing the genetic activity of chemical substances.

A diploid sacharomyces cerevisiae strain, D7 developed by Zimmermanne et al. (1975) allows the detection of gene conversion at the gene locus trp5 by a prototroph selection technique. At the same time, this strain has also two copies of the allele ilv1-92/ ilv11-92 which enable the study of reverse mutations.

In the present work the ability of Astiban which is an anti-bilharzial drug to induce mitotic gene coversion and reversion in the yeast strain D7 was studied.

MATERIALS AND METHODS

a- Materials:

(1) Yeast strain:

The diploid strain D7 has been described by Zimmermann et al. (1957), carries two non-complementing alleles, trp 5-12 and trp 5-27 which cause a requirement for tryptophan. Prototrophy can be restored by intragenic recombination, mostly non-reciprocal gene conversion, with a mixture of reciprocal intragenic crossing-over and reverse mutations. There is a pair of complementing alleles, ade 2-119 (not causing a pink pigmentation of colonies). Both alleles are recessive and D7 forms white colonies. However, mitotic crossing-over, mitotic gene conversion and rare events of point mutation, chromosomal deletion or loss of an entire chromosome can lead to the expression of one of the two alleles. This can readily be detected by the appearance of pigmented colonies. Only reciprocal mitotic crossing-over can be recognized as such by the appearance of colonies with simultaneously occurring red and pink sectors. All other types of colonies can be caused by any of the above mentioned genetic events. Therefore, all pigmented colonies are combined into one category which is said to reflect mitotic segregation. There is also a homozygous condition for allele ilu 1092 which causes a requirement for isoleucine. Restoration of prototrophy can be brought about by mutation of the mutant allele itself or in some suppressor genes.

(2) Media:

i. Yeast extract peptone glucose broth (YEPG):

This medium was used for routine culture growth, consisting of: 1% Difco yeast extract, 2% Difco bactopeptone, 2% glucose as carbon source and water up to 100 ml.

ii. Yeast extract peptone glucose agar (YEPG agar):

This solid medium was used also for routine culture growth by adding 2% Difco agar-agar to YEPG medium.

iii. Synthetic minimal medium:

This medium was used for experimental treatments.
It consists of:

$(NH_4)_2SO_4$	10.0 g
KH_2PO_4	8.75 g
K_2HPO_4	1.25 g
$Mg\ SO_4.7H_2O$	5.00 g
Na cl	1.00 g
$H_3\ BO_3$	0.1 ml 0.1% solu
$Cu\ SO_4.5H_2O$	0.1 ml 0.1% solu
KI	0.1 ml 0.1% solu
$Fe\ Cl_3.6H_2O$	0.1 ml 0.5% solu
$Zn\ SO_4.7H_2O$	0.1 ml 0.7% solu

All these ingredients were dissolved in a final volume of 1 Litre. This solution is then used as the stock solution. Hundred ml stock solution was supplemented with 1 ml of (10% $CaC_2.2H_2O$); 1 ml vitamin solution contains (0.2 mg Biotein, 40.0 mg Thiamin, 40.0 mg Pyridoxine, 200.0 mg Inositol, and 40.0 mg Capantothenate dissolved in 100 ml water). 2% glucose and 2% agar-agar. The mixture was completed to 1000 ml with distilled water (Zimmermann, 1975).

iv. Synthetic complete medium:

It is composed of synthetic minimal medium supplemented with, 5 mg/L L-tryptophan.

v. Selective medium:

This medium was used for preparing selective agar plates to identify convertant colonies and reverse colonies. It was composed of all the components of the synthetic complete medium except tryptophan (screening for convertants), or except isoleucine (screening for reverse mutants) (Zimmermann, 1975).

b- Methods:

D7 cells were inoculated to 3 test-tubes each containing 5 ml of liquid yeast extract peptone glucose broth (YEPG). The tubes were mixed well and incubated for 24 hours at 30 C. Homogeneous 0.1 ml samples were transferred to a series of tubes each containing 0.5 ml of the same medium (YEPG) supplemented with Astiban with the concentrations of; 0, 0.2, 0.4, 0.6, 0.8, 2.0, 4.0, 6.0, 8.0, 20.0, 40.0, 60.0 and 80.0 µl. Each tube was completed to 1.4 ml volumes, with sterile distilled water before the addition of 0.1 ml yeast cell suspension. The tubes were mixed well and incubated in a shaking water bath for 18 hours at 30 C. The tubes were freezed for at least one hour to stop all biological reactions. Samples of 0.1 ml with suitable dilutions from each treatment were spread on yeast extract peptone glucose agar (YEPGA) and on selective media supplemented with:

1. 5 mg/l L-addenine
2. 30 mg/l L-isoleucine
3. 10 mg/l L-tryptophan

The plates were incubated at 30°C for 5 days. Then they were scored and colonies were counted.

RESULTS AND DISCUSSION

Table (1) gives the mean numbers of cells survived Astiban concentrations on YEP and supplemented selective media, their percentages, numbers and percentages of reversions (able to grow without isoleucine) and mitotic gene conversions (able to grow without tryptopha) in two completely separate experiments.

Table (1) and Figures (1 and 2), showed that Astiban had different lethality effects for different doses used. Table (1) showed that the percentages of survival ranged from 54.6% to 13.0% in the first experiment, but in the second experiment it ranged from 68.6% to 6.7%. The dose of 60 µl Astiban/1.5 ml was the highest toxic dose in experiment 1 while the dose of 4 µl/1.5 ml was the highest toxic dose in experiment 2. Moreover, in addition to different toxicity of Astiban in the different experiments, its toxic effect is not dose dependent. The lowest toxic dose in both experiments were 0.4 µl/1.5 ml. In both experiments the dose 0.4 µl/1.5 ml stimulated growth, the results of other concentrations varied in their toxic effects. No mutagenic effect appeared in experiment 1 as no reversions for isoleucine were obtained. However, reversions were obtained in experiment 2 in four doses; (4.0 µl) which gave the highest reversion percentage was the highest toxic dose (Table 1). The percentages of conversions (tryptophan independent) in experiment 1 ranged between 0% (dose 80.0 µl/1.5 ml) to 2.5% (dose 4.0 µl/1.5 ml) but in experiment 2 it ranged between 0.32% (dose 80.0 µl/1.6 ml) to 3.27% (dose 4.0 µl/1.5 ml). This indicates that the dose 0.4 µl/1.5 ml gave the lower toxic, while the dose 4 µl/1.5 ml gave highest percentages of reversions in experiment 2 and the highest conversions in both experiments 1 and 2 while in experiment 1 the highest toxic dose was 60 µl/1.5 ml.

These two experiments indicate that Astiban is mutagenic or probably carcionogenic at least in some doses.

Table 1: Mean numbers of D7 cells survived Astiban concentrations on YEP and supplemented selective media, their percentages, number and percentages of reversions and mitotic gene conversions.

Astiban conc./ µl/1.5 ml	No. of cells/ml on YEP(x1000)	%	No. of cells/ml on supple. selective M.(x 1000)	%	No. of reversion /ml	%	No. of conversion /ml	%
			EXPERIMENT NUMBER ONE					
0.00	190.1±51.3	100	318.9±31.7	100	0	0	0.00	0
0.20	80.6±19.9	42.4	122.1±40.5	38.3	0	0	0.4±0.31	0.33
0.40	102.6±20.0	54.6	153.2±50.4	48.1	0	0	1.0±0.72	0.65
0.60	80.0±21.4	42.1	117.0±46.9	36.7	0	0	0.7±0.67	0.61
0.80	76.6±22.6	40.3	112.9±30.0	35.4	0	0	0.7±0.48	0.62
2.00	83.0±13.5	43.7	134.0±41.2	42	0	0	0.4±0.21	0.31
4.00	60.8±22.0	32	96.0±39.2	30.1	0	0	2.4±1.27	2.5
6.00	87.7±35.2	46.1	136.9±54.0	42.9	0	0	0.3±0.20	0.59
8.00	97.3±35.1	51.2	137.5±45.6	43.1	0	0	1.6±1.44	1.16
20.00	86.9±35.2	45.7	136.1±53.9	42.7	0	0	0.6±.33	0.44
40.00	57.5±18.3	30.3	81.3±17.3	25.5	0	0	0.1±.06	0.13
60.00	24.6±10.4	13	68.6±21.6	21.5	0	0	0.1±.10	0.15
80.00	33.3±47.8	17.5	73.8±15.6	23.2	0	0	0.0±0.0	0
			EXPERIMENT NUMBER TWO					
0.00	208.6±32.3	100	240.1±71.3	100	0.00±0.00	0	0.00±0.00	0
0.20	132.6±38.5	63.6	60.7±34.1	25.3	0.03±0.03	0.05	0.43±0.26	0.71
0.40	143.0±36.6	68.6	72.1±40.5	30	0.00±0.00	0	0.87±0.29	1.21
0.60	88.0±40.1	42.2	60.0±35.1	25.1	0.00±0.00	0	0.83±0.61	1.38
0.80	75.4±64.8	36.1	56.0±30.3	23.3	0.00±0.00	0	0.77±0.35	1.38
2.00	87.4±6.6	41.9	57.9±29.1	24.1	0.10±0.10	0.1	0.63±0.63	1.1
4.00	14.0±4.8	6.7	43.7±22.0	18.2	0.33±0.33	0.76	1.43±0.09	3.27
6.00	22.0±2.6	10.6	54.0±21.3	22.5	0.00±0.00	0	0.57±0.38	1.06
8.00	50.3±4.0	24.1	51.8±27.3	21.6	0.00±0.00	0	1.17±1.18	2.26
20.00	44.8±6.7	21.5	48.7±22.5	20.3	0.10±0.10	0.21	0.17±0.09	0.35
40.00	36.5±11.8	17.5	48.2±23.2	20.1	0.00±0.00	0	0.37±0.37	0.77
60.00	32.7±11.2	15.7	46.8±21.2	19.5	0.00±0.00	0	0.33±0.24	0.71
80.00	59.4±16.2	28.5	52.7±26.0	21.9	0.00±0.00	0	0.17±0.17	0.32

TABLE 1

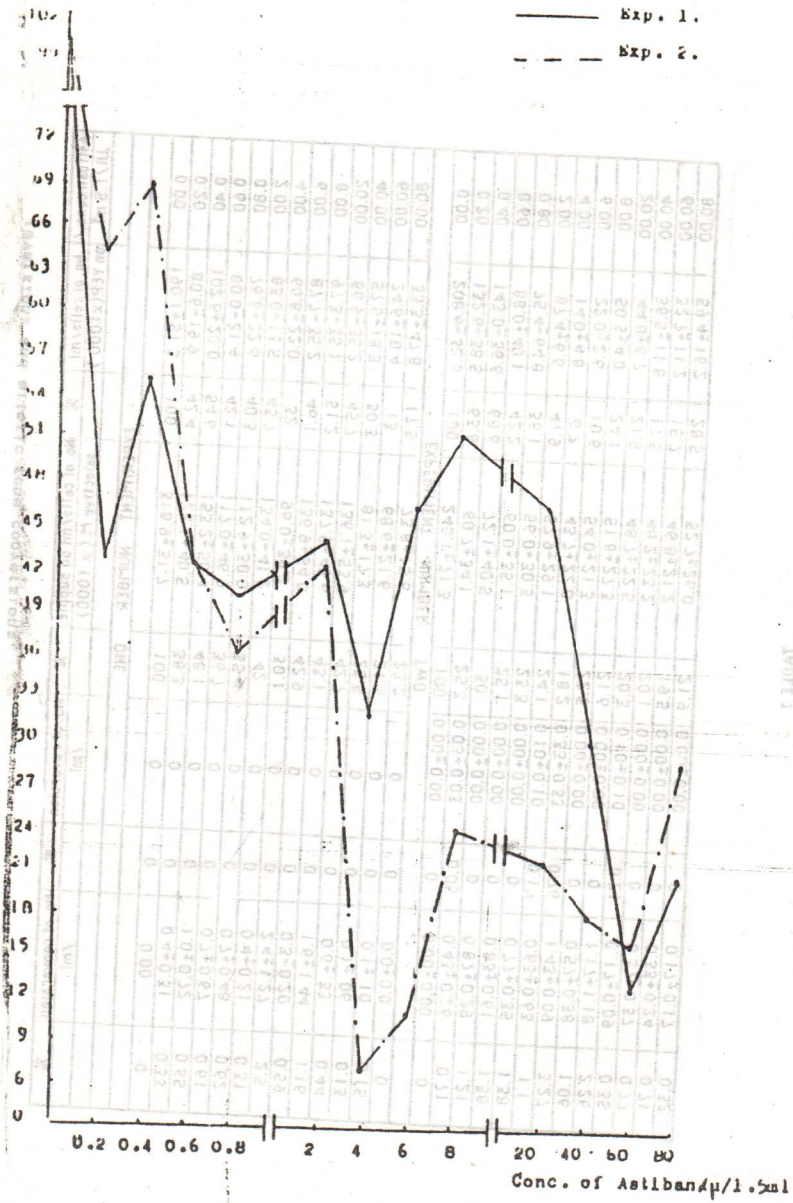

Fig.1 : D_7 yeast cells survived different Astiban concentrations in two experiments.

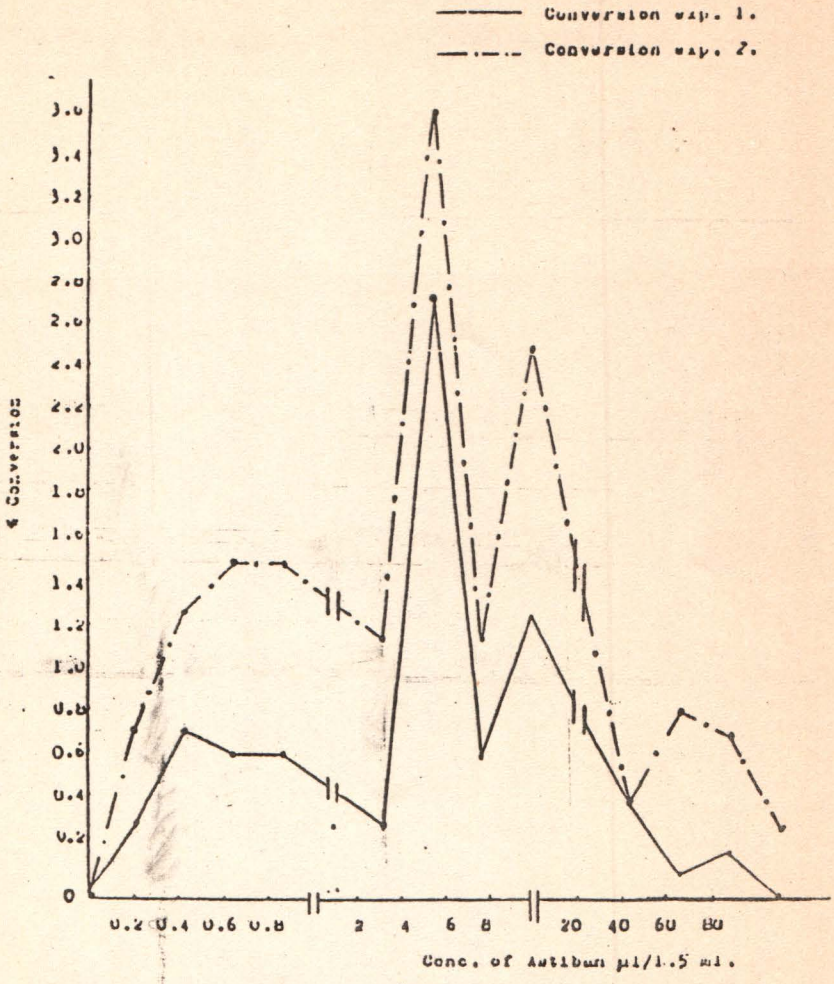

Fig. 2 H_7 yeast cells conversions percentages following Antiben treatments.