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# Resistance acquisition after seven cycles of in vitro Bleomycin exposure

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## Introduction

Bleomycin is an antineoplastic drug that causes DNA damage by intra- and inter-strand crosslink and DNA strand breaks. This drug is used to treat several types of cancers, including lymphoma, squamous cell carcinoma of the cervix, head, and neck, and Hodgkin\'s disease. Resistance to bleomycin is a major concern in the clinic. Although the cellular and molecular mechanism of resistance to this drug is not entirely clear, there are several possibilities for that this phenomenon can occur: alteration of the cellular entry and exit of bleomycin, higher repair capacity of DNA damage induced by Bleomycin and increase in bleomycin inactivation metabolism.

## Objectives

The aim of this work is to study the effect of Bleomycin on *S. cerevisiae* cells and select bleomycin-resistant cells to study the process of resistance acquisition to establish new protocols in *in vitro* drugs exposure.

## Methodology

### Yeast strain and culture medium

The experiments were carried out with the haploid yeast strain *Saccharomyces cerevisiae* WS8105-1C (*MATalpha, ade2, arg4-17, trp1-289, ura3-52*). Yeast cells were grown in a solid medium of YPD for the cytotoxicity assay, and in a liquid medium of YPD for selecting bleomycin-resistant cells of this organism.

### Chemicals

The antineoplastic drug used was bleomycin. The doses used were 0, 0,001, 0,003, 0,005, 0,008, 0,01, 0,03 y 0,06 UI/ml for the cytotoxicity assay and for selecting bleomycin -resistant cells was 0,05 UI/ml in cycle 1-5 and 0,158 UI/ml (ID 90) in cycle 6-7.

### Experimental protocol

**Cytotoxicity test:** cells were added to test tubes with different doses of bleomycin and they were completed with sterile water. Then, the tubes were cultured during 24 hours at 30°C and cells washed twice with sterile water. For drop test assay, six 10-fold serial dilutions from each sample were prepared and five-microliter aliquots of each dilution were spotted onto YPD plates.

**Resistance selection:** cells were added to test flask with liquid medium of YPD and 0.05 UI/ml of bleomycin in cycle 1-5 and 0.158 UI/ml (ID 90) of bleomycin in cycle 6-7. Then, the flask were cultured during 15 days in orbital shaking at 300 rpm and at 30°C. Next, a reseeding was carried out in another test flask with the same conditions. The process was carried out seven times (seven cycles). The OD600 was measured daily.

## Results and conclusions

The cytotoxicity curve for bleomycin obtained by drop test showed that the surviving fraction decreased gradually as bleomycin dose increased in the wild strain *S. cerevisiae* (WT). The ID50 and ID90 values obtained were 0.001 UI/ml and 0.158 UI/ml, respectively.

When WT was exposed to bleomycin doses, in the different resistance cycles, it was obtained that in cycle 1 and for a N value of 1.2E+8 cells/ml the stationary phase was reached on day 14.

In cycle 2 and for a N value of 9E+7 cells/ml the stationary phase was reached on day 7. In cycle 3 and for a N value of 1.1E+8 cells/ml the stationary phase was reached on day 6. In cycles 4 and 5 and for a N value of 1.1E+8 cells / ml the stationary phase was reached on day 5. In cycles 6 and 7 and for a N value of 1.25E+8 cells/ml the stationary phase was reached on day 6.

In addition, there was a delay in cell growth. So, for a value of N of 4E+7 cells/ml cycle1 presented a time of 9 days, cycle 2 of 6 days, cycle 3 of 5 days and cycles 4-7 of 2 days approximately. Thus it was observed that for the same value of N, as more cycles were done, the time to reach that value decreased.

The 7 cycles lasted 110 days. Cycle 1 that for a N value of 1.1E+8 cells/ml presented a time of 15 days, cycles 2-6 that for a N value of 1.1E+8 cells/ml presented a time of 30, 47, 60, 80 and 96 days, respectively, and cycle 7 which, for a N value of 1.3E+8 cells/ml, presented a time of 110 days.

To confirm that the yeast acquired resistance, the same cytotoxicity test was carried out. The decrease of the surviving fraction in the WT was clearly greater than in strain WS8105-1C-R 0.158 Bleo (resistant strain). In addition, in the resistant strain the ID50 and ID90 values obtained were 0.0156 UI/ml (15.6 times more resistant with respect to the WT) and 2.818 UI/ml (17.83 times more resistant with respect to the WT), respectively.

In conclusion, at first cells were sensitive to the drug and had low cell growth, but when cells were subjected to drug concentration in each cycle, they developed resistance mechanisms that allowed them to survive, grow and divide in the presence of bleomycin, thus increasing cell growth. In addition, it could also be observed that the stationary phase was reached in a shorter time. These facts indicate the acquisition of resistance in only 7 cycles of exposure. The results indicate that continuous exposure in only 2-7 cycles are enough to obtain high bleomycin resistance, suggesting that new protocols of *in vitro* exposure should take into account in future studies.

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