



# Determination of Sensitivity Pattern of DNA Repair Genes to Anticancer Drugs



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## Abstract

Cancer chemotherapeutic agents are known to interact with DNA and damage it in several ways. The study was aimed to determine the sensitivity pattern of DNA repair gene to two anticancer drugs namely Bleomycin and Spermidine. The haploid strain BY4741 of *Saccharomyces cerevisiae* and null derivatives of Ung, apn1, apn2, adk adk1, imp2', pho8o, pho8l, pho85 were obtained from Euro scarf Research centre United Kingdom and used for the study. The mutant cells were inoculated on the solid Yeast Peptone Dextrose (YPD) agar media treated with 60µl of the agents and 8ml of 20mg/ml of antibacterial agent, then incubated at 30 °C for 48 hours. The finding of the study showed that the parent strain (wt) is resistant to both agents by showing similar cells growth to that of control except in few mutants, suggesting that damage cause by the agents is fully repaired. The result of this research proposed that Bleomycin and Spermidine can be use as anticancer agent as its damage to cell cannot be repaired by most DNA repair genes. It's also indicates that SPD is more potent than BLM, as some genes that can repair BLM damage but cannot repair SPD damage.

**Keywords:** Bleomycin; Cancer; DNA; Mutant; Spermidine

**Abbreviations:** DNA: Deoxyribose Nucleic Acid; SPD: Spermidine; BLM: Bleomycin; YDP: Yeast Peptone Dextrose; RNA: Ribose Nucleic Acid

## Introduction

Cancer is assumed to be a new disease by many, but it has been recognition for millennia in human [1]. Cancer is one of the killer worldwide [2]. At moment, cancer accounts for 7 to 8 million deaths (13% of all deaths) worldwide [3]. In spite of constant campaigns to defeat cancer, such as Nixon's War on Cancer, all have failed, because cancer is not a single disease [4]. In fact, it is a pool of multifarious diseases characterized by loose cell multiplication that can arise from contributions from several dissimilar causes, comprising genetic and environmental [5]. The management of cancer is place on the use of chemotherapeutic drugs to eliminate cancer cells, reduce tumor growth, and relieve pain [6]. Chemoprevention remains the strategy for prevention of cancer, which means the application of natural or artificially produced substances to decrease the threats cancer infection or to decrease the probability it occurrence [7]. The drug's used for cancer chemotherapy are classified as alkylating agents, ant metabolite (e.g. 5-Fluorouracil: 5FU), anti-tumor antibiotics (anthracyclins and non-anthracyclins e.g. bleomycin) [8,9].

DNA-damaging agents have a long history of use in cancer chemotherapy [10]. The complete level of their cellular mechanisms, which is crucial to equilibrate efficacy and toxicity, is repeatedly unclear [11]. Bleomycin has a lengthy history in cancer

management, as a cytotoxic agent touching various malignant tumours such as germ cell cancers, lymphomas, Hodgkin's disease, head and neck cancer, and Kaposi's sarcoma [12,13]. Polyamines are crucial for various biotic activities in prokaryotic and eukaryotic cells. However, the pool of intracellular polyamines can be naturally occurring. Proliferation of cancer cells depend on the polyamine levels, thus, interfering with vital route of polyamine metabolism may serve to inhibit tumor growth [14,15]. Spermine and Spermidine (polyamines) are low molecular weight aliphatic polycations existing in the cells of all living organisms [16]. Owing to their positive charges, Spermine and Spermidine bind to macromolecules such as DNA, RNA, and proteins [17]. They are involved in different processes comprising cell signaling modulation, regulation of gene expression, and stabilization of cell membrane' translation and cell proliferation, they also control the activities of certain sets of ion channels [18]. For all these multidimensional roles, the homeostasis of polyamines is essential and is ensured through regulation of biosynthesis, catabolism, and transport [19]. The polyamines Spermidine and Spermine are assumed to hold diverse functions in the protection of DNA from oxidizing agents [20]. However, at low quantities Spermine is pharmacologically more active than Spermidine. Seiler and Raul, [21] reported that, both low and excessive amounts of polyamines could trigger cell

death. In this research, DNA lesion chemotherapeutic agents are applied to DNA repair genes to determine their level of sensitivity to the drugs.

## Materials and Methods

### Materials

Bleomycin (Calbiochem China, Batch Number D001179), Spermidine (Calbiochem China), Yeast Peptone Dextrose (YPD) media, haploid strain BY4741 of *Saccharomyces cerevisiae*, Distilled water, Yeast, Glucose, Ampicillin and Petri dishes.

### Preparation of media

Ten gram (10g) of yeast (Fisher bio reagents, batch number Bp1422-Z) 20g of peptone (Oxoid, batch number WYLP0037) and 20g of agar (University of Wolverhampton LAB. M, batch number Q37771/345) were mixed together and dissolved in one liter (1L) of distilled water in universal container (1000ml bottle). The mixture was autoclave for 20 minutes at temperature of 210 °C, and maintained at 50 °C. 50ml of 40% glucose (Sigma Aldrich lot number STBB3514) was added to the mixture followed by addition of 8ml of 20mg/ml ampicillin as antibacterial agent.

### Preparation of plates

The plates are prepared by pouring 60ml of the media properly mixed with require concentration of the drug. A total of three plates are made, two for the drugs and a control plate. For Bleomycin and Spermidine plates, 60ml of the media was mixed each with 60µl of Bleomycin (Calbiochem China, Batch Number D001179) and Spermidine in a sterilized container separately, swirled and homogenized the mixtures and then dispensed into the petri dishes. Control plate was prepared the media into the petri dish without adding any drugs.

### Cells culture preparation

The haploid strain BY4741 of *Saccharomyces cerevisiae* and null derivatives of Ung, apn1, apn2, adk adkl, imp2', pho8o, pho8l, pho85 were obtained from Euroscarf Research centre United Kingdom. To determine the growth of the mutant cells per 100µl of each mutant, cell culture was mixed with 2ml of YPD in to sterilized tube and incubated at 30 °C for 24 hours.

### Cell dilution

Cell dilution was made based on the number of cells from the cell count, 10,000 cells/µl culture was prepare by pipetting appropriate volume of the cell and made to 200ml with YPD, the volume of cell taken was determine from the equation  $y=3x(y+100)$ . The haploid diluted were: ung1, apn1, apn2, mdtl, adkl, ipm2', pho80, pho8l, pho85 and wt. Dilutions were made to produce 3000, 1000, 300, 100, 30, 10 and 3 cells/µl from the original concentration (10,000 cells/µl).

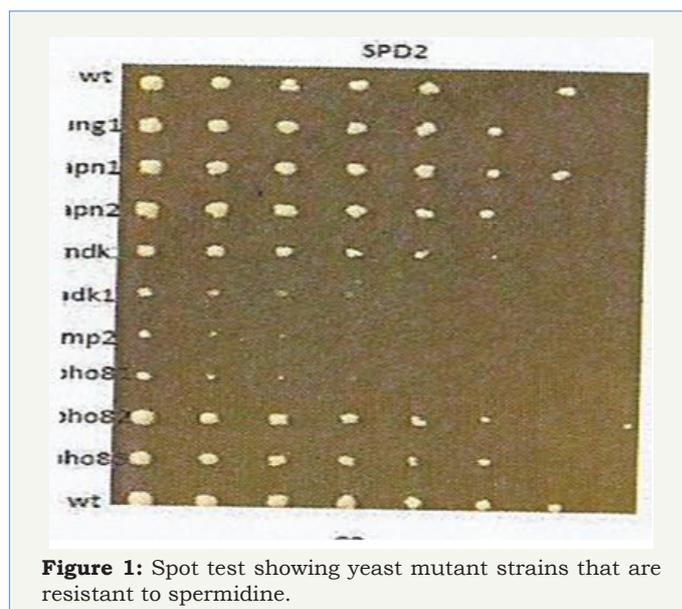
### Sensitivity test

The cells were inoculated on the treated and untreated solid YPD agar media and incubated at 30 °C for 48 hours. Sensitivity of the mutant cell to the drugs was observed after 48 hours [15]. The

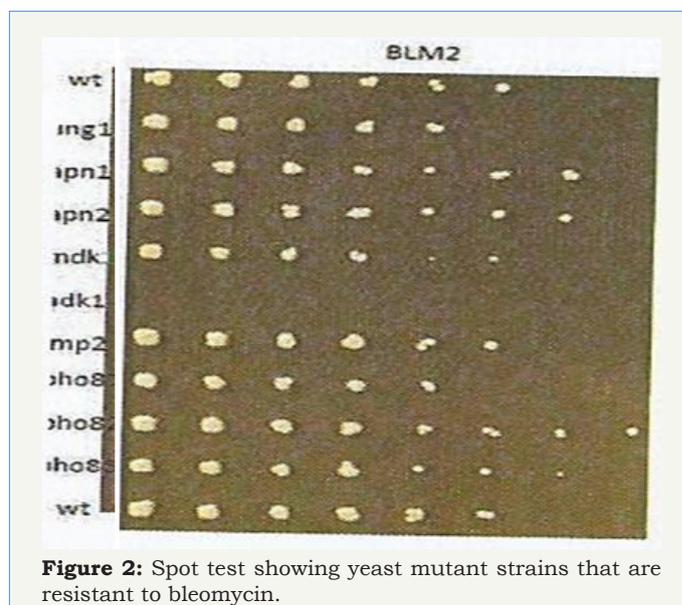
exponentially growing cultures have been diluted by 3-fold dilution, spotted onto solid YPD media and photographed after 48 hours of growth in an incubator at 30 oC. Wild type (wt) is the parent strain from where all other mutants are derived.

## Results and Discussion

The photograph of sensitivity of yeast mutant strains against cancer chemotherapeutic agent Bleomycin and Spermidine was examined. The cells were treated with different concentration of the drugs to determine the strength with optimum activity and the respond to agents. The photographic nature of the mutant's sensitivity to drugs is presented in Figure 1 & 2. The control experiment without any drug is presented in Figure 3.



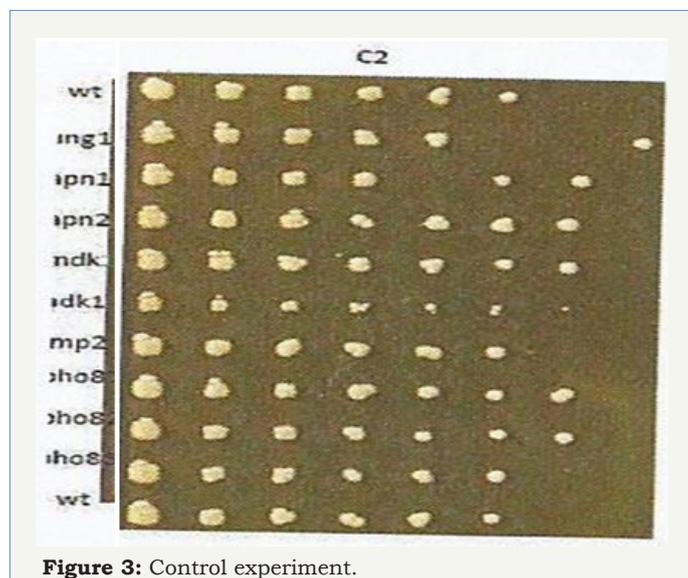
**Figure 1:** Spot test showing yeast mutant strains that are resistant to spermidine.



**Figure 2:** Spot test showing yeast mutant strains that are resistant to bleomycin.

The Figure 1 labeled SPD2 contained YPD treated with Spermidine, BLM2 Figure 2 is Bleomycin treated plate, while C2 Figure 3 is a control containing no agent. Wild type (wt) is the parent strain in which no protein is knocks out. However, in the

other cells a particular protein (DNA repair gene) is knock out as per labeled. The result of this research indicates that it is resistant to both agents (SPD and BLM) by showing similar cells growth to that of control except in few mutants, suggesting that damage cause by the agents is fully repaired.



**Figure 3:** Control experiment.

In Figure 1 (SPD) *ung1*, *apn1*, *apn2*, *pho82* and *pho85* has parallel sensitivity with wt, meaning these genes are not responsible for the cell damage cause by this agent, while *adk* mutants show mild sensitivity to the agent, suggesting its participation in the repair of damage cause by the agent. On the other hand, *adk1*, *imp2* and *pho80* are highly sensitive to SPD. No growth is seen with these mutants, suggesting cell replication is halted and the damage is not repaired. *Pho82* mutant is totally resistant SPD damage as normal cell replication continued after 48 hours of exposure to the agent. In comparison *adk1* and *pho82* mutants, they showed similar hypersensitivity and total resistivity to the activity of both agents. However, wt, *ung1*, *apn1*, *apn2*, *adk*, *pho80* and *pho85* show similar mild sensitivity to both agents. Almost all the mutants have similar sensitivity to both SPD and BLM as suggested by Ramotar [15] that mutants' sensitive to BLM are also sensitive SPD. A deviation from Ramotar's suggestion is seen with *imp2* and *pho80* mutants who are hypersensitive to SPD partially sensitive to BLM.

The result of this research proposed that SPD can be use as anticancer agent as its damage to cell cannot be repaired by most DNA repair genes. It's also indicates that SPD is more potent than BLM, as some genes that can repair BLM damage but cannot repair SPD damage. With BLM plate only *adk1* is hypersensitive for total stoppage of cells growth. All other mutants show mild sensitivity except *pho82* that is totally resistant to the agent as normal cell growth continued. Spermidine is involved in different processes, comprising cell signaling modulation, regulation of gene expression, and stabilization of cell membrane' translation and cell proliferation. It also controls the activities of certain sets of ion channels [18]. Therefore, hypersensitivity mutants were associated with an increased accumulation of polyamines. Knocking out the gene coding this enzyme will lead to accumulation of polyamine

and hence cell death [22]. Conclusively, the Spermidine kills cells when over express or accumulated by lack of proper metabolism. Parallel to this assertion my result shows almost all mutants sensitive to Bleomycin are equally sensitive to Spermidine as reported by Siham et al. [23]. This could be due to a defect in the extrusion of toxic levels of polyamines, raising the possibility that a similar defect could be associated with BLM toxicity. Aouida et al. [24] suggested that the BLM and polyamine phenotypes might have same functional defect.

### Conclusion

In the present study, a mutant cell of *Saccharomyces cerevisiae* and its null derivatives were tested against two anti cancer drugs (Bleomycin and Spermidine) to determine the pattern of their sensitivity to the drugs. The finding of the study showed that the parent strain (wt) is resistant to both agents by showing similar cells growth to that of control except in few mutants, suggesting that damage cause by the agents is fully repaired. The result of this research proposed that Bleomycin and Spermidine can be use as anticancer agent as its damage to cell cannot be repaired by most DNA repair genes. It's also indicates that SPD is more potent than BLM, as some genes that can repair BLM damage but cannot repair SPD damage.

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