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Anti-cancer Activity of the Microorganism *Saccharomyces boulardii* in Probiotics

Aishwarya R1, Jenifer Joseph2
1 Department of Biotechnology, Prathyusha Engineering College, Thiruvallur
Email - aishraga96@gmail.com
2 Department of Biotechnology, Prathyusha Engineering College, Thiruvallur

**ABSTRACT**

Cervical cancers, a common cancer that affect women, have around 500 new cases coming up every year. Although if detected early, it could be cured, chances are that it is still fatal. A few research studies claim that probiotics can fight against cancer, especially colorectal cancer. But not a lot of work has been done when it comes to cervical cancer. The aim of this study was to find out whether *Saccharomyces boulardii*, a microorganism found in probiotics, can inhibit the cervical cancer cells. The probiotics sample was taken in an eppendorf tube inside which 1ml of dimethyl sulfoxide was added along with PBS buffer and serially-diluted. Meanwhile, HeLa cancer cells (1 × 10^5/well) were plated in 24-well plates and incubated in 37°C with 5% CO₂ condition. Once the cells reached confluence, the sample was added and incubated for 24 hours under appropriate conditions. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4). 100µl/well of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide (MTT) was added and incubated for 4 hours. 1ml of DMSO was added in all the wells to dissolve formazan product formed due to the MTT assay. The control was set up as a mixture of the cancer cell, MTT solution, and the DMSO. The absorbance at 570nm was measured using spectrophotometer. The half maximal inhibitory concentration (IC₅₀) of the sample was found to be 7.8 g/ml, suggesting that approximately half the cancer cells were inhibited when the concentration of the probiotics was 7.8 g/ml. This makes *Saccharomyces boulardii* a potential anticancer agent against cervical cancer.

**KEY WORDS:** Probiotics, Cervical cancer, MTT Assay

**INTRODUCTION**

Cervical cancer is the second most common cancer that affects women, after breast cancer. In India, about 130,000 cases come up every year out of which 70,000 to 75,000 deaths occur [1, 13]. It is caused by Human Papillomavirus (HPV), which forms warts in the throats and genital area [3]. It has been established that HPV infection is the central cause of Invasive Cervical Cancer (ICC). The sexually transmitted genital HPVs are the etiologic factor in cervical cancer worldwide [4, 5].

Some of the causes of cervical cancer include early marriage, having multiple sexual partners, undergoing multiple pregnancies, and excessive smoking. Cervical cancer does not occur in a few days. It happens gradually, over a period of time, though a series of four stages – The first stage is the transmission of HPV, followed by its viral persistence. Then, the clone is progressed which turns he infected cells into precancers. Finally, invasion occurs and the cervical cancer begins to form [17].

For experimental purposes, an immortal cell line called HeLa cell line is used. It was named after Henrietta Lacks, a patient who died of cervical cancer in the year 1951. The doctor, George Gey, was able to isolate one specific cell, multiply and start a cell line [15]. In vitro studies showed that some drugs and chemicals that inhibit HeLa cells have the potential to fight against the real cancer. Now, not only drugs, but even extracts from medicinal plants like Thai showed anti-proliferative activity on HeLa cell line [11].

Probiotics are defined as “live microorganisms” that are believed to provide health benefits when consumed [2]. The word “probiotic” has been derived from the Greek term “biotikos”, which is literally translated as “for life”. The microorganisms which serve as probiotics are *Lactobacillus sp.*, *Streptococcus sp.*, and even species of yeasts including...
Saccharomyces cervisiae and Saccharomyces boulardii.

Prebiotics have been used for many years in the animal feed industry. But, times have changed and these days, they are made available as a supplementary diet for human consumption and it is present in many forms – powdered form (freeze-dried), capsules, and liquid [6]. Human body contains trillions of bacteria, especially in the gut region. In order to keep those “good bacteria”, prebiotics are used. It is not easy for them to enter the gut. The biggest barrier is the stomach, due to its acidity. But some species like Lactobacillus acidophilus survive in the acidic conditions of gastric juice at pH 3.0 at 37°C [7]. Prebiotics is a non-digestible food ingredient that is slightly different from the way probiotics work. The host is benefitted by prebiotics because it is selectively stimulated by one bacterium or a group of bacteria in the colon with prebiotic properties. Both prebiotics and probiotics are together called as Synbiotics. Studies have suggested that synbiotics allow certain changes in the gut microbiome and therefore, it is possible to prevent colon cancer [9, 10].

Cell-based assays are used to detect if the test molecules have effects on cell proliferation or show cytotoxic effects that eventually lead to cell death. Tetrazolium reduction is a typical method for detecting viable cells. The compounds used are MTT, MTS, XTT, and WST-1. Out of these compounds, MTT is the most reliable compound that can be used [16].

MTT is a water soluble dye that is yellow in colour. It is taken up by viable cells and reduced when mitochondrial succinate dehydrogenase enzymes act on them. As a result, a water insoluble product called formazon is formed. It is purple in colour. Formazones has to be dissolved for calorimetric measurement [12]. There are a few chemicals which are used to achieve this – ethanol, isopropanol, mineral oil, and DMSO.

The amount of formazone product is directly proportional to the number of living cells present during MTT exposure [18]. Colorimetric assays are measured using spectrophotometer. MTT assay is no different. The range of the light is kept between 540-720nm [14].

When there is a decrease in the cell number, it indicates the inhibition of cell growth. The concentration of a drug or chemical that inhibits 50% of the growth when compared to that of the growth of untreated control, it is called as Inhibitory Concentration, IC₅₀ [20].

There is one major advantage and a disadvantage in MTT assay. The advantage being that this assay is not only used to measure cytotoxicity and cell proliferation but also to measure cell activation. The cell activation can be measured independently of proliferation [8]. The disadvantage is that MTT assays are not always reliable and accurate. When the cells that are cultured increase the superoxide formation, MTT assay yields inaccurate results. This is because superoxides also reduced tetrazolium salts to produce the absorbed formazone end products [21]. Nevertheless, MTT assay is still used in various techniques.

Saccharomyces boulardii is a yeast which is closely associated to Saccharomyces cervisiae. It is extensively used to treat a wide variety of GI tract disorders. It has also been reported that this yeast prevented colon cancer colony formation, reduced cell proliferation and induced apoptosis [9]. But what is unclear is whether this microorganism has a role in cervical cancer too and whether it can stop the proliferation of HeLa cells and turns out to be a new anticancer agent. This is what our study aims to find out.

**EXPERIMENTAL PROCEDURE**

**Cell Line and Culture**

HeLa cell line was obtained from Veterinary College, Vepery, Chennai. The cells were maintained in Minimal Essential Medium (MEM) supplemented with 10% Foetal Bovine Serum (FBS), penicillin (100U/ml), and streptomycin (100 g/ml) in a humidified atmosphere of 50 g/ml CO₂ at 37°C.

**Reagents and Materials**

MEM was purchased from Hi Media Laboratories. FBS was purchased from Cistron Laboratories. MTT and DMSO were bought from Sisco Research Laboratory Chemicals, Mumbai, and other chemicals were bought from Sigma Aldrich, Mumbai.

**Preparation of Sample**

A known quantity of the powdered probiotics containing Saccharomyces boulardii was taken in an eppendorf tube to which 1ml of dimethyl sulfoxide (DMSO) and PBS buffer were added. PBS buffer is a non-toxic buffer and it was added to maintain the pH and provide the aqueous sample an essential nutrient, phosphorous. This was serially-diluted to reduce the density of the culture to a more usable concentration. HeLa cell
line (1x10^5/ well) was placed in a 24-well plate and it was incubated in 37°C with 5% CO_2 condition.

When the cells reached 80% of confluence, the prepared sample was added and incubated for 24 hours. After incubation, the sample was removed from each of the wells after which they were washed with PBS (pH 7.4).

MTT Assay
3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide (MTT) Assay was performed by following Mosmann, 1983. In this method, 100 g/ml of MTT was added in each well and incubated for 4 hours. After incubation, 1ml of DMSO was added in all the wells. Using a UV Spectrophotometer, the absorbance at 570nm was noted by sing DMSO as the blank solution. IC_{50} and the cell viability percentage was calculated using the following formula:

\[
\text{Cell Viability Percentage} = \left( \frac{A_{570 \text{ of treated cells}}}{A_{570 \text{ of control cells}}} \right) \times 100
\]

Where, A570 is the value of absorbance at 570nm.

Graph was plotted with Cell Viability % on Y-axis and the Concentration on X-axis. Cell control and sample control was included in each assay to compare the full cell viability assessments. Table 1 shows the values obtained from the spectrophotometer and the corresponding graph is shown in Figure 1.

### Table 1 – Cell Viability Percentage

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Dilutions</th>
<th>Absorbance (O.D)</th>
<th>Cell Viability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>Neat</td>
<td>0.228</td>
<td>21.13</td>
</tr>
<tr>
<td>500</td>
<td>1:1</td>
<td>0.244</td>
<td>22.61</td>
</tr>
<tr>
<td>250</td>
<td>1:2</td>
<td>0.280</td>
<td>25.94</td>
</tr>
<tr>
<td>125</td>
<td>1:4</td>
<td>0.308</td>
<td>28.54</td>
</tr>
<tr>
<td>62.5</td>
<td>1:8</td>
<td>0.408</td>
<td>37.81</td>
</tr>
<tr>
<td>31.2</td>
<td>1:16</td>
<td>0.430</td>
<td>39.85</td>
</tr>
<tr>
<td>15.6</td>
<td>1:32</td>
<td>0.486</td>
<td>45.04</td>
</tr>
<tr>
<td>7.8</td>
<td>1:64</td>
<td>0.511</td>
<td>47.35</td>
</tr>
<tr>
<td>Cell control</td>
<td>-</td>
<td>1.079</td>
<td>100</td>
</tr>
</tbody>
</table>

**Figure 1 – Concentration vs. Cell Viability Percentage**

**RESULT**

From the graph, it is clear that as the cell viability reaches around 50% when the concentration is 7.6 g/ml. That is, when the percentage is exactly at 47.35, it is taken as IC_{50}.

At this concentration, 47.35% of the cells are viable or living which is why they were able to reduce the MTT dye into its insoluble form, formazon.

Out of 100%, the remaining percentage of cells, 52.65% are not living.

**CONCLUSION**

Despite being a simple experiment, the value this holds is very large. The usage of *Saccharomyces boulardii* as an anticancer agent against cervical cancer is a novel idea because that organism has been generally used to be thought as an anticancer agent against colorectal cancer as it is a probiotic.

The absorbance values of the sample are lower than that of the control cell, indicating that there is a reduction in cell proliferation.

More than 50% of the cells are dead when the concentration of the probiotics stood at 7.6 g/ml. This is attributed to the fact that the activity of the probiotics has inhibited the HeLa cells. Hence, they are not viable anymore.
We firmly believe that *Saccharomyces boulardii* has a great potential to be an anticancer agent, especially against cervical cancers.

REFERENCE


