Results of Low $\beta$- and $\gamma$ Irradiation Doses on Saccharomyces Cerevisiae Fermentation Process

Received for publication, December 12, 2014
Accepted, June 06, 2015

DAN CHICEA$^{1,2}$, VOICHITA GHEOCA$^1$, ECATERINA LENGYEL$^3$
1-Environmental Sciences Department, University Lucian Blaga of Sibiu, Romania
2-Pediatric Respiratory Medicine Research Center (CCMRP), University Lucian Blaga of Sibiu, Romania
3-Biotechnology Department, University Lucian Blaga of Sibiu, Romania
*Address correspondence to: Environmental Sciences Department, University Lucian Blaga of Sibiu, Dr. Ion Ratiu Str. 7-9, Sibiu, 550012, Romania, dan.chicea@ulbsibiu.ro

Abstract

Four different strains of Saccharomyces cerevisiae yeast samples were $\beta$- and $\gamma$ irradiated using a $^{90}\text{Sr}$ nuclear source. The samples were irradiated for five hours acquiring a 12 Gray irradiation doses. The irradiated and non-irradiated samples were subject to a fermentation process conducted at 28°C and pH=5.8 for 96h. The fermentation parameters (carbon dioxide level and maltase activity) were recorded during the fermentation. For all the irradiated samples a significant increase in the fermentation parameters was recorded, as compared to the non-irradiated ones. The results of this ongoing study reveal that the small irradiation doses used in the work reported here produced significant increase of the fermentation parameters.

Keywords: Saccharomyces cerevisiae, $\beta$- and $\gamma$ radiation small doses, fermentation, maltase activity

Introduction

Yeasts are eukaryotic microorganisms classified as Fungi. Approximately 1500 yeast species of yeasts have been described so far (1). Yeasts have been widely used by men for alcohol related brewing and fermentation since millennia, as archaeological evidences reveal (2, 3, 4). The main agent in modern fermentation processes is the Saccharomyces complex (5) which contains some of the most important species for the food industry, among them, S. cerevisiae is the agent of wine, bread, beer and sake fermentations. The importance of S. cerevisiae has although exceeded its historical use in food industry, as new technologies are developed and new outcomes in bioengineering and genetics placed it as a model for eukaryotic biology (6). S. cerevisiae became the preferred microbial cell factory for major industrial biotechnology products and an important resource to be exploited for diverse chemical production (7). The increasing interest in bioengineering of lipids for use in functional foods, pharmaceuticals, and biofuels had brought again into attention Saccharomyces cerevisiae, as a valuable alternative (8, 9).

Industrial yeasts are of special interest for microbiology and biotechnology because of their use in alcoholic fermentations. Glycolysis, the metabolic pathway that converts glucose into pyruvate, is the first major step of fermentation or respiration in every living cell (10). In yeast the anaerobic catabolic pathway (fermentation), decompose the glucose to alcohol and
carbon dioxide. The preferred sugar is the glucose, but other sugars can be used from a substrate as sucrose and maltose.

Maltose is the main fermentable sugar in brewer's wort (around 60% of the total), followed by maltotriose (around 25%), and glucose (around 15%). Maltose fermentation is the result of two groups of enzymes: the permease, which help the passage of sugars across the cell membrane and the maltase, which hydrolyze the sugar to glucose. The production of maltase, which catalyzes the splitting of maltose in two glucose molecules, is normally inhibited by the presence of glucose in the medium (11). The use of maltose does not begin until the glucose is removed by fermentation, therefore the maltase activity is induced by the consumption of glucose. The fermentation efficiency depends on the yeast capacity to break down the most abundant sugar in the substratum, the maltose in the case of brewer’s wort.

During the last decades, ionizing radiations have been investigated to determine their influence on living organisms. Radionuclides are released into the environment from various sources: nuclear accidents, discharges from the nuclear power industry, disposal of radioactive waste, medical use, nuclear weapons development or recycling. Ionizing radiations are able to cause toxically and genetic effects on organisms, because radionuclides do accumulate in biotic and abiotic components of the environment (12). Nuclear radiation can stimulate morphogenetic changes manifest in the early development stages (13, 14, 15). Nuclear radiation can directly disturb metabolic processes, such as photosynthesis, growth, plant respiration, active transport as well as ionic balance and enzyme synthesis (16). The literature reveals that low doses of ionizing radiations can stimulate cell proliferation, (17, 18).

In this study, we investigated the low doses of $\beta^-$ and $\gamma$ radiation influence on four *Saccharomyces cerevisiae* strings, mainly the influence on the fermentation process. The details of the samples irradiation and the results of the fermentation process analysis are presented further on.

### Materials and methods

#### 1.1. Irradiation setup and procedures

The samples were irradiated one at a time in an irradiation chamber that was built for this purpose. The hole in the upper part fits a glass tube than can be easily inserted and extracted. The tube is used to place the sample in the proximity of the beta irradiation source. The schematic of the irradiation chamber is presented in figure 1.

![Figure 1. The beta-irradiation chamber](image)
The dose debit through the glass tube, in the very location where the yeast samples were placed one by one, was measured using a RFT - KD27012 dosimeter with an ion chamber. The $\gamma$ source was $^{90}\text{Sr}$ and decays by the disintegration scheme:

$$^{90}\text{Sr} \rightarrow^{\beta} Y, T_{1/2} = 28.79 \text{y}$$

having $E_{\gamma} = 546$ keV, with a branching ratio of 100% (19). The daughter nucleus, $^{90}\text{Y}$, is unstable as well. It decays by the scheme:

$$^{90}\text{Y} \rightarrow^{\beta} Zr, T_{1/2} = 64.00h$$

having the energies, branching ratios and half-lives presented in table 1.

<table>
<thead>
<tr>
<th>Radiation type</th>
<th>E, keV</th>
<th>I %</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta$</td>
<td>93.83</td>
<td>0.0000014</td>
</tr>
<tr>
<td>$\beta$</td>
<td>519.39</td>
<td>0.0115</td>
</tr>
<tr>
<td>$\beta$</td>
<td>2280.1</td>
<td>99.9885</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>1760.7</td>
<td>99.9999986</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>2186.242</td>
<td>0.0000014</td>
</tr>
</tbody>
</table>

The irradiation chamber and the nuclear $\beta^+$ radiation source were the same as those used in the previous work reported in (14, 15).

### 2.2. Biologic Materials

Four strings of *Saccharomyces cerevisiae* yeast samples were used. The first string, labeled SCP, was separated from Turkish yeast having the trademark Pakmaya. The second string was labeled SCO and was separated from yeast having the trademark Dr. Oetker. The third string, labeled SCSL, was separated from French yeast having the trademark Saff Levure. The fourth string, labeled SCH, was separated from Dutch yeast having the trademark Hollandia.

Two samples of each string were prepared, having a suffix 1 for the control, nonirradiated samples, and 2 for the irradiated samples. The yeast sample type, irradiation time and irradiation doses are presented in Table 2.

<table>
<thead>
<tr>
<th>Nr.</th>
<th>Sample</th>
<th>Irradiation time, h</th>
<th>Irradiation doses, Gray</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SCP1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>SCP2</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>SCO1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>SCO2</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>5</td>
<td>SCSF1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>SCSF2</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>7</td>
<td>SCH1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>SCH2</td>
<td>5</td>
<td>12</td>
</tr>
</tbody>
</table>
2.3. Fermentation setup and parameters

Both the control and the irradiated samples were cultivated in Malt Agar. Malt Agar is used for isolating and cultivating yeasts and molds from food and for cultivating yeast and mold stock cultures (20, 21, 22). Malt Agar contains malt extract which provides the carbon, protein and nutrient sources required for the growth of microorganisms. Agar is the solidifying agent. The acidic pH of Malt Agar allows for optimal growth of molds and yeasts while restricting bacterial growth.

The eight samples described above were subject to a fermentation process conducted in identical conditions, in an ECONOMY 20 fermenter. The temperature was maintained constant at 28°C. The acidity was maintained at pH=5.8. The maltase activity, measured in mgs of glucose produced in one hour from 100 g maltose as a result of 1 ml of enzyme extract, and the CO₂ emission was monitored for 96 hours (23).

Results and discussions

The results of the fermentation activity, measured as CO₂ emission and the maltase activity measured at 24 hours interval are presented in table 3. The CO₂ emission at 24 hours interval is presented in figure 2 and the maltase activity in figure 3.

<table>
<thead>
<tr>
<th>Nr.</th>
<th>Yeast string</th>
<th>CO₂-24h, g/l</th>
<th>maltase activity 24h, mg of glucose/ (100 ml of biomass*h)</th>
<th>CO₂-48h, g/l</th>
<th>maltase activity 48h, mg of glucose/ (100 ml of biomass*h)</th>
<th>CO₂-72h, g/l</th>
<th>maltase activity 72h, mg of glucose/ (100 ml of biomass*h)</th>
<th>CO₂-96h, g/l</th>
<th>maltase activity 96h, mg of glucose/ (100 ml of biomass*h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SCP1</td>
<td>0.5</td>
<td>780</td>
<td>1.5</td>
<td>810</td>
<td>1.3</td>
<td>800</td>
<td>0.3</td>
<td>760</td>
</tr>
<tr>
<td>2</td>
<td>SCP2</td>
<td>0.8</td>
<td>1220</td>
<td>1.6</td>
<td>1240</td>
<td>1.5</td>
<td>1200</td>
<td>0.5</td>
<td>1200</td>
</tr>
<tr>
<td>3</td>
<td>SCO1</td>
<td>0.7</td>
<td>840</td>
<td>1.3</td>
<td>850</td>
<td>1.1</td>
<td>830</td>
<td>0.2</td>
<td>820</td>
</tr>
<tr>
<td>4</td>
<td>SCO2</td>
<td>0.9</td>
<td>1280</td>
<td>1.7</td>
<td>1290</td>
<td>1.5</td>
<td>1280</td>
<td>0.4</td>
<td>1250</td>
</tr>
<tr>
<td>5</td>
<td>SCSL1</td>
<td>0.6</td>
<td>760</td>
<td>1.4</td>
<td>780</td>
<td>1.2</td>
<td>750</td>
<td>0.3</td>
<td>750</td>
</tr>
<tr>
<td>6</td>
<td>SCSL2</td>
<td>0.7</td>
<td>1190</td>
<td>1.5</td>
<td>1210</td>
<td>1.3</td>
<td>1160</td>
<td>0.3</td>
<td>1180</td>
</tr>
<tr>
<td>7</td>
<td>SCH1</td>
<td>0.7</td>
<td>860</td>
<td>1.4</td>
<td>920</td>
<td>1.1</td>
<td>900</td>
<td>0.4</td>
<td>850</td>
</tr>
<tr>
<td>8</td>
<td>SCH2</td>
<td>0.8</td>
<td>1230</td>
<td>1.6</td>
<td>1240</td>
<td>1.4</td>
<td>1220</td>
<td>0.2</td>
<td>1220</td>
</tr>
</tbody>
</table>

Examining Table 1, figures 2 and 3 we notice that the fermentation process produced by the irradiated samples (batch having the suffix 2) is more intense, which is proved by the increased CO₂ emission and by the increased maltase activity. We can also conclude that for the all four Saccharomyces cerevisiae yeast strings the relatively low 12 Gray β⁻ irradiation doses had a stimulating effect in respect of the fermentation process. The SCO and SCH strings exhibit the highest stimulation effect.

These results are consistent with previous work done on Zea mays seeds (5, 6) which proved that doses lower than 1 Gy have a stimulating effect on the length of the plantlets. It appears that bigger doses, in the range of several Gys, have a stimulating effect on Saccharomyces cerevisiae yeasts and have an inhibitory effect on Zea mays plantlets growth. This result is consistent with the results reported in (15), as well, which states that the yeast survival rate after absorbing very high β⁻ irradiation doses appears to be unchanged. The productivity increase might be caused by the antibacterial effect of the β⁻ and γ irradiation.
This explanation is consistent with the results reported in (25), where much bigger $\gamma$ irradiation doses proved to be an efficient treatment to decontaminate the cane must previously inoculated, not the yeast.

![Figure 2](image1.png)
**Figure 2.** The CO$_2$ emission (in g/l) for the four *Saccharomyces cerevisiae* yeast strings.

![Figure 3](image2.png)
**Figure 3.** The maltase activity for the four *Saccharomyces cerevisiae* yeast strings

**Conclusions**

The results reported in this work suggest a procedure for improving the productivity of *Saccharomyces cerevisiae* yeasts. The procedure is quite simple and consists of irradiating the samples with nuclear radiation at relatively low doses, in the range of several Gys.
Results of Low $\beta$- and $\gamma$ Irradiation Doses on Saccharomyces Cerevisiae Fermentation Process

References

14. D. CHICEA, M. RACUCIU, On the effects of low doses (0 – 1.2 Gy) beta radiation on Zea mays seeds on 12 days plantlets, Romanian Journal of Physics, 52, 5-6, 663 - 640, (2007).