Chapter 4

STUDIES ON PRODUCTION OF ACETIC ACID BY ALCOHOL AND TEMPERATURE RESISTANT STRAINS OF Saccharomyces cerevisiae Y_B

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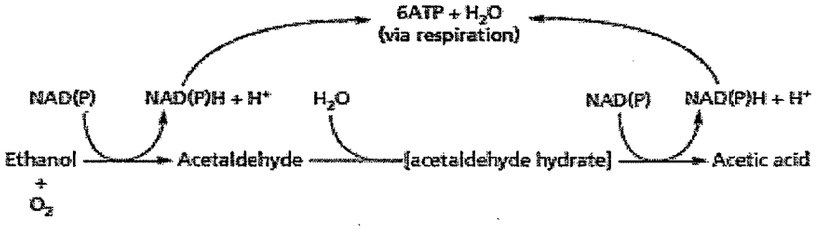
The acetic acid fermentation is a highly aerobic process, essentially a biotransformation by acetic acid bacteria, involving incomplete oxidation of ethanol to acetic acid. This fermentation in an oxidative process in which diluted solutions of ethanol are oxidized by atmospheric oxygen to acetic acid & water. Other minor products include acetaldehyde, ethyl acetate and acetoin. The quantity of the byproducts produced depends on the medium composition, the yeast strain & the fermentation step (330-332). The oxidation proceeds according to the following equation:



Pampulha et al. (333) have worked on the interaction of the effects of acetic acid & ethanol on inhibition of fermentation in *Saccharomyces cerevisiae*. They found that, acetic acid inhibited fermentation in a mutant of *Saccharomyces cerevisiae* (IGL-3507-111) deficient in respiration in an exponential rate. The undissociated form of the acid probably was the toxic agent. Ethanol potentiated the effect in a synergistic exponential way.

Romano, P. et al (334) investigated 96 strains of apiculate wine yeasts for their ability to produce higher alcohols & acetic acid in synthetic medium. Among them, *Kloeckera apiculata* produced twice as much acetic acid as *H. guilleimondii*. The production of higher alcohols & acetic acid was found to be a characteristic of individual strain & was statistically significant.

Ciani, M. & Ferraro, L. (335) showed that the oxygen concentration exerted strong influence on both growth & acetic acid production by *Brettanomyces* yeast in winemaking. Full aerobiosis leads to a large production of acetic acid blocking

metabolic activities. Semi-aerobiosis resulted in the best condition for alcoholic fermentation (Custers Effect) combined with acetic acid production. In anaerobic condition, *Brettanomyces* yeasts did not result in high acetic acid production; rather a pure, even if slow, alcoholic fermentation occurred.

Erasmus, D. (336) compared each one of the seven commercially available wine yeast strains for icewine production. He found that, *Saccharomyces cerevisiae* ST produced the lowest amount of acetic acid & glycerol, while the strain VIN7 produced the highest amount of acetic acid & glycerol. Global gene expression analysis revealed that, genes involved in glycerol & acetic acid formation were expressed at higher levels in VIN7 than in ST.

Casey, E. et al (337) examined the effect of acetic acid on the cofermentation of glucose & xylose under controlled pH conditions by *Saccharomyces cerevisiae* 424A (LNH-ST), a genetically engineered industrial yeast strain. The presence of acetic acid in the fermentation media led to a significant decrease in the observed maximum cell biomass concentration. The ethanol production rate also decreased when acetic acid was present but ethanol metabolic yields increased under the same conditions.

The main objective of the present work is to investigate the production of acetic acid by using ethanol & temperature resistant strains of *Saccharomyces cerevisiae* Y_B.

MATERIALS & METHODS:

Microorganism: The microorganisms used are some of the alcohol & temperature resistant strains of *Saccharomyces cerevisiae* Y_B.

Medium & Cultural conditions: The medium used for the production of acetic acid contained- Sucrose-12.0%, KH_2PO_4-0.1%, $(NH_4)_2SO_4$-0.5%, yeast extract-0.1% and $MgSO_4$, $7H_2O$- 0.05%. pH was adjusted to 4.5. This solution was sterilized at 121°C & 15lb/inch^2 pressure for 15 minutes. The yeast cells were harvested by washing the slants with sterile distilled water. The cell density was adjusted to 2.05×10^5 cells/ml. 5ml of cell suspension was used for the inoculation of the fermentation medium. Fermentation was carried out using 250ml Erlenmeyer conical flasks, each containing 100ml of the medium & incubating them at 30°C for 48hours.

Estimation of acetic acid: Concentration of acetic acid was determined by High Performance Liquid Chromatography (HPLC) with a TPS Spectra System apparatus using a Biorad Aminex HPX-87H column heated to 40°C and a Refraction Index Detector (Waters 640). The mobile phase was sulfuric acid (0.005 M) flowing at 0.4ml/min (338).

RESULTS & DISCUSSION:

To study the production of acetic acid we inoculated the production medium separately by some alcohol & temperature resistant strains of *Saccharomyces cerevisiae* Y_B, such as- Y_{B19} to Y_{B30} (resistance to 7.5% alcohol). Table 2 indicates the production of acetic acid by the above mentioned studies-

Table 2. Production of acetic acid by alcohol & temperature resistant strains of *Saccharomyces cerevisiae* Y_B :

STRAINS	ACETIC ACID (gm/100ml)
Y_{B19}	**0.1527±0.007**
Y_{B20}	**0.1501±0.001**
Y_{B21}	**0.1347±0.005**
Y_{B22}	**0.1385±0.002**
Y_{B23}	**0.1562±0.004**
Y_{B24}	**0.1323±0.002**
Y_{B25}	**0.1232±0.006**
Y_{B26}	**0.1269±0.005**
Y_{B27}	**0.1293±0.001**
Y_{B29}	**0.1433±0.003**
Y_{B30}	**0.1263±0.004**

Production values are expressed as mean ± SD.

All values are biologically significant ($p<0.001$).

From Table 2, we can see that, all the ethanol & temperature resistant strains of *Saccharomyces cerevisiae* Y_B can produce acetic acid from ethanol. But the strain *Saccharomyces cerevisiae* Y_{B23} produces maximum amount of acetic acid (0.1562 gm/100ml). So we used this strain for our further studies.