

**Chapter 2**

***SELECTION OF THE MEDIUM FOR THE  
MAINTENANCE OF ALCOHOL AND  
TEMPERATURE RESISTANT  
Saccharomyces cerevisiae Y<sub>B23</sub>***

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### SELECTION OF THE MEDIUM FOR THE MAINTENANCE OF ALCOHOL AND TEMPERATURE RESISTANT *Saccharomyces cerevisiae* Y<sub>B23</sub>

Microorganisms require sufficient amount of energy to maintain their life activities. As *Saccharomyces cerevisiae* is a chemotrophic microorganism, it receives its energy from several chemical nutrients, such as: C, N, S, P, Na, K, Ca, Mg, Fe, Mn, Zn, Cu, Co, Mb, several vitamins & minerals, to keep on its metabolic activities in proper order, which will promote its growth & biological functioning. So, we must keep in mind, the cultivation of microorganism must be done in such a medium, which can supply sundry nutrients in sufficient amount. We know that a synthetic medium is a chemically defined media composed of known pure chemical substituents. So, the resistant strain *Saccharomyces cerevisiae* Y<sub>B23</sub> which is resistant to ethanol & temperature is to be grown & subcultured in a definite synthetic medium, so that its alcohol producing efficiency remains constant. In the present study, we observed that the said strain isolated from 7.5% alcohol & 28-30°C temperature, degenerated to show decreasing alcohol producing efficiency, when grown in YPD agar slant & subcultured in the same medium for 12 months. Thus, for better maintenance of *Saccharomyces cerevisiae* Y<sub>B23</sub> & better alcohol production, the following three media were tested for their suitability. Their compositions are given below-

Composition of Medium I	Composition of Medium II	Composition of Medium III
Glucose - 1.0%	Peptone - 0.5%	Glucose - 1.0%
Yeast Extract - 0.5%	Yeast Extract - 0.5%	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> - 0.5%
Peptone - 0.5%	Beef Extract - 0.3%	KH <sub>2</sub> PO <sub>4</sub> - 0.1%
Agar - 4.0%	Agar - 4.0%	MgSO <sub>4</sub> ·7H <sub>2</sub> O - 0.025%
pH - 5.0	pH - 5.0	FeSO <sub>4</sub> ·7H <sub>2</sub> O - 0.002%
		Biotin - 0.2µg/ml
		Agar-4.0%
		pH - 5.0

When the *Saccharomyces cerevisiae* Y<sub>B23</sub> strain was maintained in those three media separately & alcohol production was measured, we got the following results as in Table 1.

**Table. 1. Alcohol production by *S.cerevisiae* Y<sub>B23</sub> maintained in different medium:**

Maintenance medium	% of Alcohol produced								
	Initial	1 month	2 month	3 month	4 month	6 month	8 month	10 month	12 month
<b>I</b>	3.4±0.01	3.4±0.03	3.0±0.02	2.7±0.01	2.0±0.04	1.2±0.005	0.7±0.003	0.3±0.001	0.0±0.0
<b>II</b>	3.4±0.02	3.4±0.01	3.2±0.05	2.6±0.03	2.1±0.01	1.6±0.008	1.3±0.006	1.0±0.01	0.8±0.004
<b>III</b>	3.4±0.03	3.4±0.03	3.4±0.02	3.4±0.04	3.4±0.02	3.4±0.04	3.4±0.04	3.4±0.03	3.4±0.05

Production values are expressed as mean ± SD.

All values are biologically significant (p<0.001).

**Table.1.** shows that the alcohol production (%) by the alcohol and temperature resistant strain *Saccharomyces cerevisiae* Y<sub>B23</sub> subcultured in **medium III** for 12 months is relatively stable in comparison with the two other maintenance media (**medium I & medium II**). So medium III was finally selected for the maintenance of the alcohol and temperature resistant strain *Saccharomyces cerevisiae* Y<sub>B23</sub>.