INTERNATIONAL JOURNAL OF DECCAN PHARMA AND LIFE SCIENCES

www.ijdpls.com

(Original Article)

Received: 01-08-2012; Revised; Accepted: 09-08-2012

ROLE OF MACRO AND MICRO MINERAL ELEMENTS ON ETHANOL PRODUCTION BY AN ETHANOL RESISTANT STRAIN OF *SACCHAROMYCES CEREVISIAE* AB910X1

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Keywords:

Experimental, Macro and micro minerals, ethanol, *Saccharomyces cerevisiae*

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ABSTRACT

An experimental study was carried out on the effect of different macro and micro mineral elements on cellular growth, invertase activity and ethanol production by an ethanol resistant strain of *Saccharomyces cerevisiae* AB910X1.Production was maximum with $MgSO_4$. $7H_20$, 0.05% ; KH_2PO_4 , 0.15 % and $ZnSO_4.7H_2O$,15µg/ml.Other minerals namely NaCl ,$CaCl_2$, $FeSO_4.7H_2O$,$CuSO_4.5H_2O$ and $MnSO_4.4H_2O$ showed adverse effect on cellular growth, invertase activity and ethanol production.

INTRODUCTION

Similar to carbon and nitrogenous compounds, inorganic metal salts are important nutrients for growth and metabolite production by different microorganisms. However, requirements of nutrients vary with type of microorganisms and the basal media used under investigations. Considerable studies have been made on the requirements of minerals for growth and ethanol production by different yeasts. Besides appropriate carbon and nitrogen sources, yeasts' growth depends upon compounds containing phosphorus, potassium, magnesium, sulfur and traces of zinc, iron and copper[1].

Reviews claimed that several mineral elements showed either positive or negative impacts on ethanol production by different strains of yeasts[2-12].

Considering the reviews our present study was intended to examine the role of different mineral elements on cellular growth, invertase activity and ethanol production by an ethanol resistant mutant *Saccharomyces cerevisiae* AB910X1 and subsequently select a suitable synthetic medium for ethanol production by this resistant strain.

MATERIALS AND METHODS

Microorganism: Yeast *Saccharomyces cerevisiae*AB910X1 used in this study was isolated from North Bengal pineapple waste disposal materials, then it was resistant to temperature, ethanol.

Maintenance of the culture: Yeast culture was maintained in YPD agar medium (dextrose, 1.0 %; peptone, 0.5%; yeast extract, 0.5 %; agar, 4.0 % and pH 5.0)

Medium used for ethanol production: Production medium contained: sucrose, 20%; $(NH_4)_2SO_4$, 0.7%; KH_2PO_4, 0.1% and $MgSO_4.7H_2O$, 0.05%. pH was adjusted to 5.0.

Preparation of inoculum: Cells were harvested by adding sterile distilled water in the agar slant and scrapping the microorganism. Uniform cell suspension were prepared by breaking cell clumps and cell density was maintained at $10x10^5$/ml.5 ml of cell suspension was added in 100 ml production medium contained in a 250 ml Erlenmeyer conical flask. Production was carried out at 30^0C for 48h. All the Chemicals (Analytical Grade) were purchased from E.mark and triple distilled water was used throughout the study.

Determination of cellular growth: Cellular growth was estimated by spectrophotometer using 600nmwave length[4].

Determination of ethanol concentration: Ethanol concentration was determined according to the method as described by Stackler *et al*.(1974)[13].

Determination of invertase activity: Invertase activity was determined according to the method as described by Vitoly *et al.*(1991)[14].

Statistical analysis: Data were presented as the mean of at least three independent experiments along with SEM. Student t test was employed considering $p<0.001$ using MS Excel.

RESULTS AND DISCUSSION

The effects of different macro and micro mineral elements were depicted in Table 1 and Table 2 respectively.

Table 1: Effects of macro-mineral elements on growth, invertase activity and ethanol production by *Saccharomyces cerevisiae*AB910X1

Macro-mineral element(s)	Concentration(s)[%]	Dry cell weight(gm/L)	Invertase activity(Unit/ml)	Ethanol production(%)
NaCl	0.00(control)	6.0±0.012	73.0±1.134	8.4±0.011
	0.03	5.1±0.042	65.8±1.087	7.6±0.231
	0.05	5.0±0.063	60.2±1.087	7.5±0.132
	0.10	4.9±0.058	47.3±1.090	7.1±0.361
	0.25	4.7±0.091	45.3±1.043	6.7±0.011
	0.50	4.6±0.011	42.3±1.065	6.0±0.056
$MgSO_4.7H_2O$	0.03	5.2±0.032	62.5±1.523	7.8±0.098
	0.04	5.5±0.072	65.4±1.533	8.1±0.053
	0.05(control)	6.0±0.013	73.0±1.768	8.4±0.061
	0.06	5.9±0.033	71.0±1.093	8.2±0.031
	0.07	5.8±0.068	66.5±1.931	8.0±0.032
KH_2PO_4	0.05	5.6±0.081	70.3±1.023	8.0±0.043
	0.01(control)	6.0±0.093	73.0±1.343	8.4±0.056
	0.15	6.2±0.017	73.2±1.261	8.5±0.091

	0.20	5.8±0.083	71.6±1.633	8.3±0.014
	0.25	5.2±0.091	68.0±1.536	8.3±0.032
$CaCl_2.2H_2O$	o.oo(control)	6.0±0.037	73.0±1.902	8.4±0.014
	0.03	5.8±0.056	69.3±1.323	8.3±0.061
	0.05	5.8±0.051	64.2±1.209	8.3±0.071
	0.10	5.8±0.077	56.0±1.434	8.2±0.051
	0.25	5.7±0.066	55.7±1.543	8.0±0.064
	0.50	5.4±0.051	52.4±1.202	7.8±0.021

(values were expressed as mean±SEM,where n=3;all values of ethanol production were significant compared to control considering p<0.001).

Table 2: Effects of micro-mineral elements on growth, invertase activity and ethanol production by *Saccharomyces cerevisiae*AB910X

Micro-mineral element(s)	Concentration(s)[µg/ml]	Cellular growth(gm/L)	Invertase activity(unit/ml)	Ethanol production(%)
Fe^{2+} ($FeSO_4.7H_20$)	0.0(control)	6.0±0.065	73.0±1.681	8.4±0.087
	1.0	5.8±0.031	56.7±1.528	8.3±0.032
	5.0	5.7±0.054	55.6±1.633	8.1±0.071
	10.0	5.7±0.093	52.0±1.902	7.6±0.063
	15.0	5.5±0.012	48.2±1.682	6.8±0.076
	20.0	4.7±0.061	40.1±1.316	6.1±0.095
Cu^{2+} ($CuSO_4.5H_2O$)	0.0(control)	6.0±0.033	73.0±1.639	8.4±0.017
	1.0	5.1±0.012	41.2±1.281	6.3±0.003
	5.0	3.3±0.091	36.1±1.361	5.2±0.043
	10.0	1.1±0.002	21.2±1.127	4.2±0.065
	15.0	–	–	–
	20.0	–	–	–
Mn^{2+} (MnSO4.4H2O)	0.0(control)	6.0±0.041	73.0±1.192	8.4±0.082
	1.0	6.0±0.056	73.0±1.165	8.4±0.163
	5.0	5.6±0.081	54.2±1.325	8.0±0.077
	10.0	5.1±0.063	42.2±1.198	6.6±0.056
	15.0	4.6±0.012	40.0±1.423	6.3±0.061

	20.0	4.0±0.043	38.2±1.109	5.9±0.056
Zn^{2+} ($ZnSO_4.7H_2O$)	0.0(control)	6.0±0.013	73.0±1.439	8.4±0.053
	1.0	6.0±0.045	73.0±1.187	8.4±0.072
	5.0	6.0±0.091	73.0±1.166	8.4±0.038
	10.0	6.1±0.043	73.6±1.365	8.8±0.091
	15.0	6.3±0.068	73.4±1.982	8.6±0.056
	20.0	6.0±0.055	73.0±1.162	8.4±0.091

(values were expressed as mean ± SEM, where n=3;all values of ethanol production were significant compared to control considering p<0.001).

From the above mentioned Tables it is clear that, among all these mineral elements studied, production of ethanol was maximally obtained using this resistant strain with $MgSO_4.7H_2O$, 0.05% ; KH_2PO_4,0.15% and $ZnSO_4.7H_2O$,15µg/ml. Other elements exerted detrimental effects on cellular growth, invertase activity and ethanol production.

CONCLUSION

After optimizing different mineral elements, the following synthetic medium is finally recommended for ethanol production by the resistant *Saccharomyces cerevisiae*AB910X1:Sucrose,20%;$(NH_4)_2SO_4$,KH_2PO_4,0.15%,$MgSO_4.7H_20$;0.05 % and $ZnSO_4.7H20$;15µg/ml. pH was adjusted to 5.0. From the above mentioned Tables it is clear that, among all these mineral elements studied, production of ethanol was maximally obtained using this resistant strain with $MgSO_4.7H_2O$, 0.05% ; KH_2PO_4,0.15% and $ZnSO_4.7H_2O$,15µg/ml. Other elements exerted detrimental effects on cellular growth, invertase activity and ethanol production.

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