

CHANGES IN TRICHLOROACETIC ACID SOLUBLE ANTHRONE POSITIVE CARBOHYDRATE LEVEL IN *SACCHAROMYCES CEREVISIAE* S1 UNDER STRESS CONDITIONS

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Saccharomyces cerevisiae S1 inocula were prepared in PYN medium which consisted of (gl^{-1}) peptone, 35; yeast extract, 30; KH_2PO_4 20; $(\text{NH}_4)_2\text{SO}_4$, 10 and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$; with glucose 50gl^{-1} with reciprocal shaking (100rpm) for 18h at 28, 32 and 36°C . From these cultures, aliquots were transferred to a set of pre-equilibrated sterile flasks at 58°C . In another set-up 50ml culture from 28°C was transferred to 36°C , incubated for 90min and then transferred to pre-equilibrated flask at 58°C . Viability of the cultures was monitored. The cultures grown at 28, 32 and 36°C showed 1, 12 and 28% viability at 58°C after 5min. When heat shocked, (28 to 36°C) culture was subjected to 58°C , 20% viability was observed. The cells directly transferred from 36 to 58°C showed 2% viability at 10min. The rapid shift in growth temperature from 28 to 36°C has resulted in significant increase in viability (50%). Therefore, by effecting appropriate growth temperature, the thermotolerating capacity of the yeast could be enhanced. The effect of temperature shift cultivation on ethanol tolerance was studied. *Saccharomyces cerevisiae* S1 inoculum was prepared in PYN medium at 36°C with reciprocal shaking (100rpm) for 18h. The aliquots of inocula were given different treatments, which included ethanol shock (200gl^{-1} added ethanol to the medium) or heat shock at 45°C for 30min, or the combination of the both. Consequently, the cultures were grown at either 36°C or 40°C and viability of the cells was monitored. The viability of cultures, which had no treatments (heat shock or added ethanol) at either 36 or 40°C remained 100%. Heat shocked cells (at 45°C for 30min) showed 100% viability when they were grown at 40°C without the added ethanol. When 200gl^{-1} ethanol was added to the culture (18h) grown at 36°C , complete cell death was observed at 60h. When the temperature was increased from 36 to 40°C in the presence of ethanol, the time taken for the complete cell death was 60 and 30 min, respectively. Therefore, the toxic effect of ethanol was aggravated by the increase in temperature. However, the temperature-induced toxicity was nullified by a brief heat shock (30min) at 45°C . Heat shocked culture showed 37% viability at 30min as against complete cell death of the cells, which were not given heat shock at 40°C in the presence of ethanol. Effect of heat shock on thermotolerance and Trichloroacetic acid (TCA) soluble anthrone positive carbohydrate was studied. The cultures of *Saccharomyces cerevisiae* S1 grown at 36°C was given a heat shock at 45°C for 30 min, another portion of the culture was allowed to grow at 36°C without heat treatment (control). From control and heat shocked cultures 1ml aliquotes were kept at 58°C for 5min and, viability & TCA soluble anthrone positive carbohydrate contents were determined. The TCA soluble anthrone positive carbohydrate content increased by 90% in heat shocked cells, while 28% viability was observed for heat shocked cells as

against complete cell death for culture not undergone heat shock. Therefore, the increase in TCA soluble anthrone positive carbohydrate could be linked to enhanced thermotolerance of the *Saccharomyces cerevisiae* S1 cells. Since the heat stress has increased the anthrone positive carbohydrate content in the cells, the effect of ethanol stress on the anthrone positive carbohydrate content of the cells was studied. A 44% increase in anthrone positive carbohydrate content was induced by ethanol shock. Ethanol shock as well as heat shock has induced the accumulation of TCA soluble anthrone positive carbohydrate. In the mean time heat shocked cells showed better ethanol tolerance. The stress conditions have been circumvented by the production of TCA soluble anthrone positive carbohydrate.