

**Thesis Title**                      Strains Improvement of Zymomonas mobilis IFO 13756  
for Sorbitol Production

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**M.S.**                                      Biology

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**Abstract**

Strains improvement of Zymomonas mobilis IFO 13756 for sorbitol production was carried out by induced mutation using N-methyl-N'-nitro-N-nitrosoguanidine (NTG) 2,000 µg/ml for 20 min. at 30 °C. One hundred and twenty fructose negative mutants (Fru<sup>-</sup>) were isolated by ampicillin and penicillin G enrichment and replica plating method. These were screened for sorbitol production using 25% sucrose as a carbon source. The Z. mobilis CS-92 which produced and accumulated the

highest amounts of sorbitol was selected. Cell extract of Z. mobilis CS-92 was studied for fructokinase activity and separated by anionexchange chromatography. It was found that Z. mobilis CS-92 could produce fructokinase protein, but that protein had lost its functional properties.

Optimal conditions for sorbitol production of Z. mobilis CS-92 were studied. The CS-92 strains produced and accumulated maximum amounts of sorbitol 45.11 g/l in a medium containing 25% sucrose, 1% yeast extract and 0.2% of each of  $MgSO_4$ ,  $(NH_4)SO_4$ ,  $KH_2PO_4$  and bactopectone, at initial pH 6.0-7.0, at 30°C, after incubation for 48 hours.

The addition of  $FeSO_4$  and  $Na_2SO_4$  at concentrations of 10-50 mM slightly decreased sorbitol formation, but NaCl addition clearly reduced sorbitol formation and the degree of reduction increased with increasing NaCl concentration.  $CuSO_4$  and  $ZnSO_4$ , even at the lowest concentration of 10 mM, could reduce sorbitol formation completely, due to inhibition of cell growth.