

ABSTRACT

In the present study, eighty six strains of *Saccharomyces cerevisiae* were isolated from different samples of fruits and soil by serial dilution method. The strain IS-66 gave maximum extracellular invertase production (1.10 U/ml). The enzyme activity reached to 5.6 U/ml when incubation time (48 h), sucrose concentration (5 g/l) and pH 5.5 were optimized. The wild strain IS-66 was exposed to ultraviolet (UV) radiations to obtain a mutant with improved enzyme activity. UV induced mutagenesis did not produce any stable mutant and almost all of the mutants produced relatively lesser invertase than the parental strain. Strain IS-66 was further subjected to chemical mutagenesis using nitrous acid and ethyl methane sulphonate (EMS). After extensive screening, two mutants were developed with increased enzyme activity NA-45 (20.74 U/ml) and EMS-42 (34.2 U/ml) from the wild-culture (IS-66). The mutant strain EMS-42 was cultured on the medium containing 2-deoxy-D-glucose (2dg) and its stability in invertase production was determined at different concentrations of 2dg. The concentration of 0.04 mg/ml was found optimal, as at this concentration EMS-42 showed consistent enzyme activity.

Six media were evaluated for the production of invertase in shake flasks. M1 medium (g/l) containing yeast extract 3, peptone 5 and sucrose 30 g/l gave better production of invertase (25.28 U/ml) after 48 h of inoculation. Different sugars such as sucrose, glucose, fructose, lactose, galactose, maltose, raffinose and molasses were investigated on the enzyme production. Of these, sucrose was found to be best (44.03 U/ml) after optimizing the concentration at 10 g/l. Incubation temperature (30°C), inoculum size (2.0 %, v/v) and volume of the medium (50 ml/250 ml Erlenmeyer flask) were optimized. The effect of different additional nitrogen sources such as organic, inorganic and agriculture byproducts were also tested. Peptone at the concentration of 6 g/l gave maximum production of invertase (50 U/ml). The addition of inorganic nitrogen sources and agricultural byproducts nitrogen were not found to have any impact on the enhancement in enzyme production rather it was decreased from the control especially in case of agricultural byproducts. In stirred fermentor (7.5 L), the scale up studies for invertase production was carried out. The enzyme production (65.12 U/ml) was obtained after 24 h of incubation. The overall increase in enzyme activity (15 U/ml) and fermentation time was shortened by 24 h while scaling up enzyme production from shake flask to fermentor. The maximum enzyme activity (80.06 U/ml) was achieved after

optimization of cultural conditions such as sucrose (15 %, w/v), pH (4.5), inoculum size (7.5 %, v/v), agitation intensity (240 rpm) and aeration rate (1 vvm, 10 % DO).

The intracellular enzyme activity was also determined by sonication. The maximum enzyme activity (57 U/ml) was found in mutant strain of EMS-42 after 24 h fermentation in the fermentor. During sonication, the maximum specific activity of 106 U/mg of protein was obtained with 0.5 duty cycle of impulses at amplitude of 40 % and pH 5 for 60 min. The calcium alginate entrapment technique was used for immobilization of whole cells of *S. cerevisiae* EMS-42 to form inverted syrup. It was noticed that maximum sucrose hydrolysis (63.40 %) was achieved after 18 h of incubation time. By optimization of cultural conditions for sucrose hydrolysis, the maximum hydrolysis percentage (76.3 %) was obtained at 50°C, pH 5.5 using sucrose (60 %) as substrate.

An extracellular invertase was purified to homogeneity by two step purification i.e., ammonium sulfate precipitation and DEAE-Sephadex A-50. The enzyme was present in the supernatant of 85 % saturation being glycoprotein in nature. DEAE column chromatography eluted the enzyme as single active fraction at 0.2 M NaCl. The enzyme was purified by 15 fold with recovery of 38 %. The molecular mass of 110 kDa was determined after SDS-PAGE. The carbohydrate content was found to be 48 %. The intracellular invertase contains both forms of glycosylated (large) and non-glycosylated (small). The same procedure was applied for glycosylated intracellular invertase (L-form) while three purification steps were performed for non-glycosylated invertase (S-form). The L-form was purified by 19 fold with recovery of 32 %. Like extracellular invertase, the molecular weight (110 kDa) for L-form was found. Ammonium sulfate precipitation separated the enzyme (S-form) as insoluble fraction. This form of enzyme was eluted at 0.3 M NaCl using DEAE-Sephadex. A single band of molecular weight (55 kDa) was estimated after Sephadex G-50 with purification (16 fold) and recovery of 17 %. For both purified non-glycosylated and glycosylated invertase the optimum pH (5) was same whereas optimal temperature, MnCl₂ and the values of the K_m and V_{max} were found to be as 50 and 60°C, 109 and 111 %, 1.2 and 1.8 mM, 909 and 1429 U/ml/min, respectively.