ABSTRACT

Genetic improvement of the microorganism is fundamental to the success of fermentation technology. The present study deals with enhanced biosynthesis of L-Phenylacetylcarbinol (L-PAC) via improvement of a wild *Saccharomyces cerevisiae* IIBα-26 strain through random mutagenesis by ultraviolet (UV) irradiations. Minimum inhibitory concentration (MIC) values for acetaldehyde (50%) and benzaldehyde were 35 µl and 14 µl by wild strain *Saccharomyces cerevisiae* IIBα-26. Out of 117 UV isolates, 21 were acetaldehyde resistant and gave L-PAC concentrations in the range of 0.47±0.038 g/l to 2.35±0.040 g/l. When 21 UV isolates were primarily screened for their L-PAC productive profiles, five gave higher L-PAC yields i.e. IIB/UV-28 (2.28±0.012 g/l), IIB/UV-54 (1.96±0.029 g/l), IIB/UV-77 (2.18±0.021 g/l), IIB/UV-91 (2.35±0.040 g/l) and IIB/UV-101 (2.05±0.040 g/l) as compared to wild strain (1.88±0.012 g/l). These five higher L-PAC producing strains were again subjected to UV irradiation and checked for their acetaldehyde tolerance in order to screen for a more competent and efficient producer of L-PAC. Out of 5 double UV isolates, a potent mutant *S. cerevisiae* IIB/UV/V-91 showed hyper L-PAC biosynthesizing capability (2.47±0.025 g/l) and higher aldehyde tolerance as compared to wild which gave L-PAC as 1.88±0.032 g/l. As compared to wild, mutant IIB/UV/V-91 exhibits lowest values of dry cell mass, glucose consumed, however, highest value of residual sugar and final pH i.e. 0.22±0.023 g/l, 25.26±0.116 g/l, 93.78±0.124 g/l and 5.94±0.051, respectively than wild during biotransformation of benzaldehyde into L-PAC.

In the comparison of kinetic parameters, mutant strain IIB/UV/V-91 exhibited highest specific growth rate (0.42±0.010 h^{-1}) than wild (0.34±0.015 h^{-1}) after 24 hrs of incubation. The mutant also showed improved values for $Y_{x/s}$ (0.29±0.008 g dry cell mass formed/g sugar consumed), $Y_{p/s}$ (0.84±0.001 g L-PAC produced/g sugar consumed), $Y_{p/x}$ (4.93±0.012 g L-PAC produced/g dry cell mass formed), Q_p (0.76±0.004 g L-PAC produced/l/h) and q_p (0.44±0.012 g L-PAC produced/g dry cell mass formed/h) to the corresponding values of wild strain. Three different broth media i.e. YPG (yeast extract, peptone and glucose medium), CNM (Complex

nutrient medium) and MUM (Molasses, urea and magnesium sulphate medium) were tested for submerged fermentation of L-PAC by wild and mutant culture. The maximum L-PAC production by both strains was obtained in MUM medium whereas the other two media showed no productivity.

Comparative rate of L-PAC biosynthesis by wild *S. cerevisiae* IIBα-26 and mutant *S. cerevisiae* IIB/UV/V-91 was monitored in shake flask studies. Mutant gave optimum production of L-PAC after using YPG inoculum 12% (v/v) having ~240×10^6 cells/ml, 18 hr old and optimum values of pH, temperature, agitation rate and initial sugar concentration for maximum L-PAC yield were found to be 5.5, 30°C, 150 rpm and 150 g/l, respectively in MUM medium. In dosage experiments, benzaldehyde dose number, intervals and pattern for mutant was 8, 30 min and descending order, respectively at cell density ($O.D_{595nm}$ 0.3/~180×10^6 cells/ml) resulted in higher L-PAC yield. Molasses addition simultaneously with benzaldehyde does not influence biosynthesis of L-PAC. However, addition of 50% acetaldehyde along with 67 mM benzaldehyde led to enhanced L-PAC production (6.82±0.09 g/l) in shake flask (250 ml).

Scale up study on lab-scale fermentor (7.5 l) resulted in maximum yield of L-PAC (7.17±0.017 g/l) by mutant at 200 rpm agitation speed, 0.5 vvm aeration flow rate. A better L-PAC production (7.38±0.09 g/l) was might be the consequence of addition of 6% silicone oil (antifoam) at rate of 0.21 ml/l before biotransformation during scale up of L-PAC fermentation. Toluene facilitated maximum extraction of L-PAC from fermented broth (broth to solvent ratio 1:2). Biotransformed sample (fermentor) obtained from fermentation of mutant *S. cerevisiae* IIB/UV/V-91 exhibited peaks of residual benzaldehyde, benzyl alcohol and L-PAC at retention times of 2.261, 2.973 and 6.503 min, respectively. After optimization of different cultural and process parameters, mutant gave 1.5 fold higher yield of L-PAC as compared to wild. The improved L-PAC productivity and lower time course of fermentation made this work cost-effective.