ABSTRACT

Aiming at the improvement of the fungal mediated biotransformation of benzaldehyde to 1-Hydroxy-1-phenyl-2-propanone was carried outat Institute of industrial Biotechnology, Government College University Lahore. Sixty two (in total) benzaldehyde and acetaldehyde resistant yeast cultures were isolated, out of which 06 from Apple, 07 from Apricot, 06 from Banana, 04 from Guava, 11 from Mango, 14 from Peach, 09 from Pomegranate and 05 were from White Grapes collected from different fruit markets. All the cultures were screened on their biotransformation potential. The culture with code APL-2 gave maximum potential and was identified as *Saccharomyces cerevisae* with amplification of ITSI region 18S rDNA and digested with Hind III in RFLP.

Present fermentation comprises two phases i.e. growth phase and biotransformation phase. The wild culture of *S. cerevisae* APL-2 was optimised to enhance the biotransformation potential with different cell density and the age before the second phase of fermentation. Cell density of $1.2x10^8$ cell/mL was achieved after 12 hours incubation gave 149 U/g cell mass of pyruvate decarboxylase which enables the yield of L-PAC (3.28g/L). Six different growth media were optimised; the medium M-5 containing Molasses (20 brix), Urea (10 g/L), $MgSO_4$ (10 g/L), Yeast extract (4 g/L), Peptone (4.0 g/L), K_2HPO_4 (1.0 g/L) and KH_2PO_4 (1.0 g/L) were found best which were further evaluated with different molasses concentrations. Medium with 20 brix of molasses was evaluated for better biotransformation.

Effect of pH and temperature in growth phase and in biotransformation phase along with benzaldehyde dose pattern were critically analysed before mutation. It was observed that initial pH (7.0) at 30°C of growth phase with five descending doses of benzaldehyde provided the suitable conditions for L-PAC production

(4.02 g/L). The biotransformation of benzaldehyde to L-PAC increased from 4.02 to 4.59g/L when the temperature was maintained during biotransformation phase at 18°C.

The wild culture *S. cerevisae*APL-2 was subjected to germicidal ultra violet lamp, gamma radiation, nitrous acid and ethidium bromide to improve the culture survival and inactivation curve of *S. Cerevisiae* APL-2 shows the variation in the lethal percentage and the mutant frequency at different time intervals. Mutant frequency was the highest at about 90% inactivation. The mutant APL-2 ^{UV3} gave 36.6% higher yield than the wild type and also significantly gave better yield as compared to 16 other mutants showing high resistants to benzaldehyde as compared to the wild type. The process was further optimised in 7.5 L glass vessel fermenter with working volume of 5.0 L with different rate of agitation and aeration keeping other conditions constantly optimised in 250 mL shake flask. Agitation of 200 rpm and aeration of 1 L/L/m showing better condition for biotransformation, yielding 8.3g/L of L-PAC. During the addition of benzyl alcohol form 0.25 to 2.5g/L were evaluated to study its effect on the conversion of benzaldehyde to L-PAC instead of benzaldehyde to benzyl alcohol due to the presence of alcohol dehydrogenases. The addition of benzyl alcohol (2 g/L) increases the L-PAC (9.9g/L) production.